细胞外腺苷介导核苷转运体ENT1阻断cAMP/p-PKA信号通路抑制肝脏糖异生

黄林林[1] 汤子慧[1] 代梦圆[2] 葛梦潇[1] 徐薇[2] 杨冬琴[1]*

([1]复旦大学附属华山医院中心实验室, 上海 200040; [2]复旦大学基础医学院免疫学系, 上海 200030)

摘要 糖尿病的关键特征是葡萄糖和脂质代谢紊乱。目前, 糖尿病已成为沉重的全球疾病负担。越来越多的证据表明, 腺苷系统在调节胰岛素和葡萄糖平衡中发挥关键作用。腺苷是一种重要的细胞代谢调节剂, 通过激活G蛋白偶联受体和核苷转运体参与能量代谢、免疫调节、氧化应激等多个生理病理过程。然而, 腺苷在肝脏糖异生调控中的角色尚未被阐明。该文从多层面验证了腺苷通过核苷转运体(equilibrative nucleoside transporter, ENT)转运入胞内后对胰高血糖素刺激引起的肝脏糖异生通路的影响。结果显示, 在体内模型中, 外源性腺苷显著抑制小鼠血糖升高。在细胞模型中, 腺苷以剂量依赖的方式抑制肝脏糖异生进而降低葡萄糖输出水平, 且无细胞毒性。肝脏组织及细胞中ENT广泛表达, 其中1型核苷转运体(equilibrative nucleoside transporter 1, ENT1)介导了腺苷抑制的肝糖输出。此外, 腺苷介导的糖异生抑制并非依赖于AMP依赖的蛋白激酶[adenosine 5'-monophosphate (AMP)-activated protein kinase, AMPK]通路的激活。最后发现, 细胞外腺苷刺激后, 细胞内的环磷酸腺苷(cyclic adenosine monophosphate, cAMP)浓度显著降低, 磷酸化蛋白激酶A(phospho-protein kinase A, p-PKA)下游蛋白表达受抑制, 细胞糖输出能力显著下降, 该抑制作用可被ENT抑制剂有效逆转, 但不能被AK抑制剂削弱。以上结果表明, 细胞外腺苷通过ENT1转移入胞内后, 抑制AC活性, 从而抑制cAMP合成和p-PKA底物蛋白的表达, 抑制肝脏糖异生功能, 最终降低糖输出水平。

关键词 腺苷; 肝脏糖异生; 平衡型核苷转运体(ENT); cAMP/PKA; 腺苷酸环化酶(AC)

Extracellular Adenosine Inhibits Liver Gluconeogenesis via Adenosine Transporter ENT1 by Blocking of cAMP/p-PKA Signaling Pathway

HUANG Linlin[1], TANG Zihui[1], DAI Mengyuan[2], GE Mengxiao[1], XU Wei[2], YANG Dongqin[1]*

([1]*Department of Central Laboratory, Huashan Hospital Affiliated to Fudan University, Shanghai 200040, China*; [2]*Department of Immunology, School of Basic Medical Sciences, Fudan University, Shanghai 200030, China*)

Abstract Diabetes mellitus, which is characterized by disorders of glucose and lipid metabolism, has become a heavy global disease burden. Increasing evidences suggest that the adenosine system plays a key role in regulating insulin and glucose homeostasis. Adenosine is an important regulator of cellular metabolism and is involved in several physiopathological processes such as energy metabolism, immune regulation, and oxidative stress through activation of G protein-coupled receptors and nucleoside transporters. However, the role of adenosine in the regulation of hepatic gluconeogenesis has not been elucidated. This article verified the regulatory effect of adenos-

收稿日期: 2023-11-01 接受日期: 2024-01-22

国家自然科学基金(批准号: 81970506、81572336)资助的课题

*通信作者。Tel: 021-66895876, E-mail: kobesakura@fudan.edu.cn

Received: November 1, 2023 Accepted: January 22, 2024

This work was supported by the National Natural Science Foundation of China (Grant No.81970506, 81572336)

*Corresponding author. Tel: +86-21-66895876, E-mail: kobesakura@fudan.edu.cn

ine on glucagon stimulated hepatic gluconeogenesis pathway after its transfer into the cytosol via ENT (equilibrative nucleoside transporter) at multiple levels. The results showed that exogenous adenosine inhibited blood glucose elevation in mice. In a cellular model, adenosine inhibited hepatic gluconeogenesis and thereby reduced glucose output in a dose-dependent manner with minimal cytotoxicity. The ENT was widely expressed in liver tissues and cells, and the ENT1 mediated the hepatic glucose output inhibited by adenosine. Furthermore, adenosine-mediated inhibition of gluconeogenesis was not dependent on activation of the AMPK pathway. After extracellular adenosine stimulation, the intracellular cAMP concentration was significantly reduced, the expression of phosphorylated PKA downstream proteins was significantly inhibited, and the cellular glycolytic output capacity was significantly reduced, and this inhibition could be attenuated by ENT inhibitors but not by adenosine kinase inhibitors. The results showed that the transfer of extracellular adenosine into the cell via the nucleoside transporter ENT inhibited adenylate cyclase activity, which in turn inhibited cAMP synthesis and the expression of phosphorylated PKA substrate proteins, inhibited hepatic gluconeogenesis, and ultimately reduced glucose output.

Keywords adenosine; hepatic gluconeogenesis; ENT; cAMP/PKA; adenylyl cyclase

2型糖尿病(diabetes mellitus type 2, T2DM)患病率逐年攀升, 是全球亟待解决的重要公共卫生问题, 预计到2050年, 全球糖尿病患者将达13.1亿[1]。最近的流行病学统计显示, 有超过四分之一的糖尿病新发病例和五分之一的糖尿病死亡病例发生在中国[2]。2型糖尿病患者空腹状态的高血糖主要是由于肝脏胰岛素抵抗引发糖异生增加所致[3]。其中, 糖异生是维持空腹血糖的关键一环, 承担了禁食后60%以上的肝糖输出[4]。糖异生指的是在空腹状态下(即低葡萄糖水平), 非碳水化合物的前体在一系列酶连反应的精密调控下生成葡萄糖的关键能量代谢过程[5]。肝脏葡萄糖生成(包括肝糖原分解与糖异生)占内源性葡萄糖生成的90%, 而肝糖调节失衡与T2D的发生发展密不可分[6]。因此, 解析肝糖异生调控机制可能为研发有效的2型糖尿病治疗手段提供新策略。

腺苷, 即腺嘌呤核苷, 是一种在细胞内外广泛分布的核苷复合物, 在多种生理调节和病理过程中扮演重要角色, 参与多个组织的能量生成、神经元信号调控、炎症和肿瘤免疫。腺苷既可通过激活位于细胞表面且与G蛋白偶联的4种腺苷受体, 包括腺苷A1受体(A1 adenosine receptor, A1AR)、腺苷A2a受体(A2a adenosine receptor, A2aAR)、腺苷A2b受体(A2b adenosine receptor, A2bAR)和腺苷A3受体(A3 adenosine receptor, A3AR), 在细胞外发挥作用, 也可以通过浓缩型核苷转运体(concentrative nucleoside transporter, CNT)和平衡型核苷转运体(equilibrative nucleoside transporter, ENT), 精密调控细胞内外腺苷浓度, 直接参与腺苷代谢或间接影响腺苷受体的活性及表达, 继而影响多种能量代谢信号通路[7-10]。既往研究发现, 腺苷受体通过调控脑交感神经等途径参与脂肪生成、葡萄糖代谢等[11-12], 而独立于腺苷受体的ENT/CNT也在调控细胞代谢中发挥着关键作用[13]。然而, 腺苷在肝脏糖异生中的调控作用仍有待探索。本研究以原代小鼠肝细胞、人胚肾细胞HEK293T和野生型小鼠为研究对象, 探究细胞外腺苷通过1型核苷转运体(equilibrative nucleoside transporter 1, ENT1)/环磷酸腺苷(cyclic adenosine monophosphate, cAMP)/磷酸化蛋白激酶A(phosphoprotein kinase A, p-PKA)信号轴对肝糖异生的影响及分子机理。

1 材料与方法

1.1 材料

1.1.1 实验细胞株 人胚胎肾HEK293T细胞购自中国科学院细胞库。

1.1.2 主要试剂 DMEM培养基、M199培养基、胎牛血清、胰岛素、地塞米松等购自美国Thermo Fisher公司; IV型胶原酶、糖输出试剂盒、胰高血糖素、丙酮酸、腺苷和8-环戊-1,3-二甲基黄嘌呤(8-cyclopentyl-1,3-dimethylxanthine, 8-CPT)购自美国Sigma-Aldrich公司; 佛司可林(Forskolin)、*S*-(4-硝基苄基)-6-硫肌苷[*S*-(4-nitrobenzyl)-6-thioinosine, NBTI]、二甲基亚砜(dimethyl sulfoxide, DMSO)、ABT 702二盐酸盐(ABT 702 dihydrochloride, ABT 702)、9-(四氢-2-呋喃)腺膘呤[9-(tetrahydrofuran-2-yl)-9h-purin-

6-amine, SQ22.536]和双嘧达莫(dipyridamole, Dipy)购自美国Selleck公司; CCK-8试剂盒、RIPA裂解液、PMSF蛋白酶抑制剂、BCA定量试剂盒、Cocktail蛋白酶抑制剂和Trizol试剂购自上海碧云天生物技术有限公司; D-Hanks、Hanks溶液购自北京索莱宝科技有限公司; cAMP试剂盒购自美国R&D Systems公司; Phospho-PKA Substrate一抗、辣根过氧化物酶标记的羊抗鼠/兔抗体购自美国Cell Signaling公司; AMPK、GAPDH一抗购自美国Abcam公司。

1.2 方法

1.2.1 RT-PCR 在各组培养的小鼠原代肝细胞中加入1 mL Trizol试剂, 充分混匀后于室温放置5 min, 加入200 μL氯仿, 振荡至成乳浊液后, 静置10 min, 以12 000 r/min于4 ℃离心15 min。转移上层水相, 并加入250 μL异丙醇, 上下颠倒混匀后静置10 min, 然后以12 000 r/min于4 ℃离心10 min, 弃去上清。加入1 mL 75%乙醇溶液, 混匀后以12 000 r/min于4 ℃离心5 min。弃去上清后将RNA沉淀在空气中干燥5 min, 然后加入50 μL DEPC水溶解, 此为提取的总RNA溶液。随后用NanoDrop紫外分光光度仪检测RNA浓度。按照说明书的操作步骤用HiScript II Q RT SuperMix逆转录试剂盒将RNA逆转录为cDNA, 并进行PCR扩增。将PCR产物在1.0%琼脂糖凝胶上以100 V于TAE缓冲液中电泳30 min。随后将凝胶置于凝胶成像系统拍照。引物序列如下(表1)。

1.2.2 细胞培养 将含0.5 mL细胞冻存悬液的冻存细胞于37 ℃温水中水浴2 min至融化后, 转移至15 mL离心管中并添加5 mL培养液, 于800 r/min离心5 min后, 置于含10%胎牛血清、1%青霉素−链霉素的DMEM培养基中, 在37 °C、5% CO_2的条件下培养。

1.2.3 小鼠原代肝细胞的分离与培养 取6~8周龄雌性C57BL/6鼠, 禁食禁水3 h后, 予异氟烷吸入麻醉。75%酒精对小鼠进行体表消毒后, 打开腹腔, 钝性游离胃周结缔组织, 暴露门静脉后, 用留置针于门静脉处穿刺, 进针, 固定, 用D-Hanks溶液进行灌注, 直至将肝脏内血流完全灌净为止, 灌注过程可断续性用棉签按压下腔静脉离断处, 以充分充盈肝脏。换用25 mL Hanks液(含0.025% IV型胶原酶)对肝脏进行原位灌注与消化5 min, 消化期间可继续用棉签按压下腔静脉离断处, 以充分消化各叶肝脏及观察肝脏消化状态。小心分离肝脏与周围结缔组织, 并剪除胆囊。轻柔取下肝脏后置于预冷的D-Hanks液中, 轻轻清洗后, 轻柔地钝性敲碎肝组织。40 μm滤网过滤。500 r/min、4 °C离心5 min, 弃去上清, 4 °C预冷PBS清洗1次后, 用M199完全培养基(37 °C预热)重悬并计数, 将细胞稀释至5×10^5/mL铺板。37 °C、5% CO_2培养3 h后, 镜下观察细胞形态, 圆形透亮为活性较好的肝原代细胞。PBS清洗后用M199完全培养基重悬, 按实验需求铺板, 培养8 h后, 用药物处理细胞。本研究涉及的动物实验经过复旦大学实验动物科学部伦理委员会审查(批准号: 2019华山医院JS-153)

1.2.4 细胞活力测定 将小鼠肝原代细胞或者HEK293T细胞计数后以3 000/孔的细胞数铺于96孔板, 在37 °C细胞培养箱中孵育过夜。然后用腺苷以

表1 RT-PCR检测相关基因表达引物序列

Table 1 Primer sequences for RT-PCR detection of indicated genes expression

基因名称 Gene	上游引物(5′→3′) Forward sequence (5′→3′)	下游引物(5′→3′) Reverse sequence (5′→3′)
ADORA1	GTG ATT TGG GCT GTG AAG GT	AGT AGG TCT GTG GCC CAA TG
ADORA2A	TGC AGA ACG TCA CCA ACT TC	CAA AAC AGG CGA AGA AGA GG
ADORA2B	GGC TAT GAT TGT GGG CAT CT	GAC AAC TGA ATT GGC GTG TG
ADORA3	TCC CTG ATT ACC ACG GAC TC	TCC TTC TGT TCC CCA CAT TC
ENT1	CTT GGG ATT CAG GGT CAG AA	ATC AGG TCA CAC GAC ACC AA
ENT2	CAT GGA AAC TGA GGG GAA GA	GTT CCA AAG GCC TCA CAG AG
ENT3	TTG GGCT CTG TAT GGG ACT C	TTC TTC AGG ATG GGT CCA AG
ENT4	CCT CCT CGC CTT GGG TCC CTT GCT C	CTG ATG CCC ATT AGC AGC GAG AAG AC
CNT1	TTT GCA GGC ATC TGT GTG TTC CTT	GGC CAT GAC AGA GGC TGC GAT TAA
CNT2	AGG CCTGGA GCT CAT GGA AGT C	GGC TCC CAT GAA CAC CCT CTT AAG
CNT3	TTG CAT TTA AGA TCC TGC CC	CCT ATG AGT TTG GCG ACCAT
GAPDH	GTG GCA AAG TGG AGA TTG TTG	CGT TGA ATT TGC CGT GAG TG

浓度梯度(0~10 mmol/L)处理6~24 h。吸去培养基，每个孔用100 μL新鲜培养基和10 μL CCK-8检测试剂在37 °C细胞培养箱中孵育2 h。之后用酶标仪测定波长在450 nm处的吸光度(*D*)值，计算细胞活力，细胞活力(%)=(腺苷处理的细胞/未给药对照细胞)×100%。

1.2.5 丙酮酸耐受实验(pyruvate tolerance test, PTT)和胰高血糖素刺激实验(glucagon stimulation test, GST) 6至8周龄的C57/BL6雄性小鼠被随机分为两组，禁食12 h。首先给小鼠注射60 mg/kg腺苷，然后在1 h后给予丙酮酸1剂量，之后通过尾静脉采样，在初始治疗后的0、30、60、90、120、150、180和210 min测量血糖水平。对于胰高血糖素刺激实验，小鼠禁食6 h，首先注射60 mg/kg腺苷，然后1 h后给小鼠注射2 mg/kg胰高血糖素，再在初始治疗后的0、30、60、70、80和90 min测量血糖水平。

1.2.6 糖输出水平检测 将小鼠原代肝细胞和HEK293T细胞在含有糖异生底物乳酸钠(20 mmol/L)和丙酮酸钠(2 mmol/L)的Krebs-Henseleit-HEPES培养液中于37 °C培养箱中孵育6 h，按照实验计划分别添加相应实验组所需的药物如腺苷、Dipy、NBTI、8-CPT、Forskolin、SQ22.536、ABT702等后，收集上清液。将梯度稀释试剂盒提供的标准浓度葡萄糖液体用作标准曲线的测绘。每孔加入50 μL葡萄糖反应液，避光37 °C温箱放置反应30 min，每孔100 μL 6 mol/L浓硫酸中止后于Infinite200 Pro酶标仪检测波长在570 nm处的吸光度(*D*)值。计算样本上清液糖浓度。参照未添加药物处理组的糖浓度对各组进行百分比标化，即可得到每组的相对糖输出量。

1.2.7 免疫印迹 细胞收集后添加含PMSF抑制剂和Cocktail蛋白酶抑制剂的RIPA裂解液于冰上裂解30 min，以13 000 r/min、4 °C离心10 min，收集上清液通过BCA试剂盒进行蛋白定量。蛋白质与5× 蛋白质上样缓冲液混匀后于100 °C金属浴中加热15 min，取30 μg/孔总蛋白上样，120 V恒压电泳后将蛋白转移至PVDF膜上，以200 mA恒流转膜。以5%脱脂奶粉室温封闭1 h，TBST清洗后加入一抗Phospho-PKA Substrate(1:1 000)、AMPK(1:1 000)、GADPH(1:5 000)于4 °C孵育过夜，TBST清洗后二抗(1:3 000)室温孵育2 h，利用ImageQuant LAS 4000mini化学发光成像系统检测蛋白表达。

1.2.8 酶联免疫吸附实验(ELISA)检测cAMP浓度 收集细胞沉淀，用预冷PBS清洗3次，以试剂盒中的细胞裂解液重悬为1×10^7细胞/mL。以反复冻融的方式裂解细胞，700 r/min、4 °C离心10 min，取上清液。按照试剂盒说明书配制cAMP标准品和样品稀释液。每个孔中加入50 μL中和缓冲液(neutralizing buffer)。在NSB(non-specific binding)孔和B0孔加50 μL 0.1 mol/L HCl。分别取50 μL各浓度标准品溶液和样品稀释液至相应孔中，再取50 μL HRP标记的cAMP工作液至各孔中。取50 μL抗cAMP单克隆抗体工作液至各孔中。在室温下，500 r/min振荡孵育2 h。弃去液体后，用检测缓冲液(assay buffer)反复清洗所有孔4次。取150 μL底物溶液至各孔中，室温静置10 min左右。加入50 μL终止液后，使用Infinite200Pro酶标仪测量波长在450 nm处的吸光度(*D*)值，计算浓度，最后以对照组进行百分比标化得到相应浓度。

1.2.9 统计分析 本研究采用GraphPad Prism 8.0.1软件进行数据统计和图表绘制，最终结果以平均值±标准差($\bar{x}\pm s$)表示。两组之间数据分析采用独立样本*t*检验，多组间比较采用单因素方差分析，$P<0.05$表示差异具有统计学意义。

2 结果

2.1 细胞外腺苷对血糖水平的调控作用

丙酮酸耐受实验和胰高血糖素刺激实验都是经典的评估糖异生能力的检测方法[14]。我们检测了细胞外腺苷对禁食后小鼠体内葡萄糖生成的影响。结果显示，注射丙酮酸后，对照组小鼠的血糖水平升高200%，并在给药后2 h达到峰值，腺苷治疗组小鼠血糖升高幅度仅为对照组的66.7%。给药2 h后，腺苷迅速抑制了糖异生底物丙酮酸注射后诱导的血糖升高(2.0 h、3.0 h)($P<0.05$)，提示腺苷可能抑制糖异生作用(图1A)。进一步探讨腺苷对空腹小鼠胰高血糖素刺激状态的影响。结果同样显示，胰高血糖素注射后对照组血糖升高了144%，腺苷组血糖上升了115%，腺苷组血糖升高幅度仅为对照组的62.5%。和对照组相比，腺苷处理显著抑制了胰高血糖素刺激诱导的血糖水平升高 (80 min、90 min)($P<0.05$)(图1B)。

2.2 细胞外腺苷对肝糖输出的影响

肝脏是糖异生最主要的场所，提取小鼠原代肝细胞进一步探究腺苷对肝糖输出的影响。结果显示，外源性腺苷能够显著降低小鼠原代肝细胞的糖输

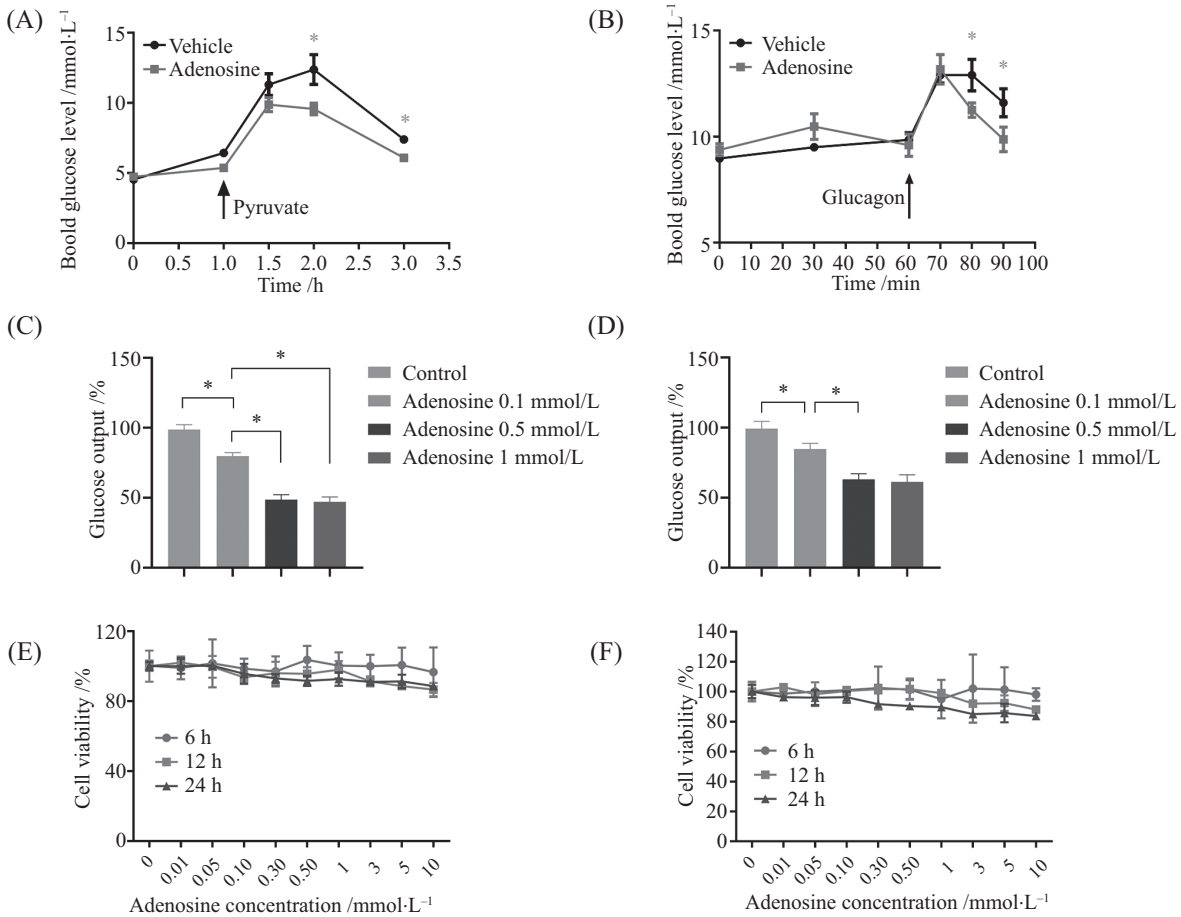
出水平(P<0.05), 且其抑制效果呈现剂量依赖性(图1C)。同时, 在HEK293T细胞模型中也得到类似结果(图1D)。CCK-8法检测细胞增殖活性发现, 腺苷引起的葡萄糖输出减少并非由细胞毒性所致(图1E和图1F)。由此可见, 细胞外腺苷在体内和体外以剂量依赖的方式抑制肝脏糖异生从而抑制葡萄糖输出, 且无细胞毒性。

2.3 腺苷受体及核苷转运体在小鼠原代肝细胞中的表达情况

细胞外腺苷主要通过激活腺苷受体或经核苷转运体进入胞内这两种途径调控下游信号通路[15]。为阐明腺苷在肝糖输出中发挥作用的可能作用途径, 通过RT-PCR检测了腺苷各个受体以及CNT和ENT等转运体家族成员的表达情况。结果发现腺苷受体A1AR微弱表达, ENT1是小鼠原代肝细胞中唯一高表达的转运体, 其余腺苷受体和转运体几乎无表达(图2A)。

2.4 核苷转运体ENT1介导腺苷抑制的肝糖输出

为明确腺苷调控血糖的作用途径, 我们在腺苷处理前用特异性A1AR拮抗剂8-CPT预处理。结



A: 体内给予腺苷(60 mg/kg)对丙酮酸耐受实验(PTT)不同时间血糖水平的影响(对照组n=4只, 腺苷组n=6只); B: 体内给予腺苷(60 mg/kg)对胰高血糖素刺激实验(GST)不同时间血糖水平的影响(n=4只/组); C: 不同浓度腺苷(0.1 mmol/L、0.5 mmol/L和1 mmol/L)作用下小鼠原代肝细胞葡萄糖输出结果(n=3); D: 不同浓度腺苷(0.1 mmol/L、0.5 mmol/L和1 mmol/L)作用下HEK293T细胞葡萄糖输出结果(n=3); E: CCK-8检测不同浓度腺苷作用下小鼠原代肝细胞活力(n=3); F: CCK-8检测不同浓度腺苷作用下HEK293T细胞活力(n=3); *P<0.05。

A: effect of *in vivo* administration of adenosine (60 mg/kg) on blood glucose levels at different times in the PTT (pyruvate tolerance test) (n=4 for vehicle group, n=6 for adenosine group); B: effect of *in vivo* administration of adenosine (60 mg/kg) on blood glucose levels at different times in the GST (glucagon stimulation test) (n=4 per group); C: glucose output results of primary mouse hepatocytes under the effect of different concentrations of adenosine (0.1 mmol/L, 0.5 mmol/L and 1 mmol/L) (n=3); D: HEK293T cell glucose output results under the effect of different concentrations of adenosine (0.1 mmol/L, 0.5 mmol/L and 1 mmol/L) (n=3); E: viability of mouse primary hepatocytes under the effect of different concentrations of adenosine detected by CCK-8 (n=3); F: viability of HEK293T cells under the effect of different concentrations of adenosine detected by CCK-8 test (n=3); *P<0.05.

图1 腺苷抑制肝糖输出和胰高血糖素诱导的糖异生

Fig.1 Adenosine inhibited liver glucose output and glucagon-induced gluconeogenesis

果显示，8-CPT处理后不影响腺苷诱导的糖输出抑制(图2A和图2B)。相反，用非特异性ENT抑制剂双嘧达莫(dipyridamole, Dipy)或选择性ENT1抑制剂NBTI处理细胞可显著逆转腺苷诱导的糖输出减少(P<0.05)(图2C和图2D)。以上结果提示，细胞外腺苷降低肝糖输出水平的作用依赖于转运体ENT1，而非腺苷受体。

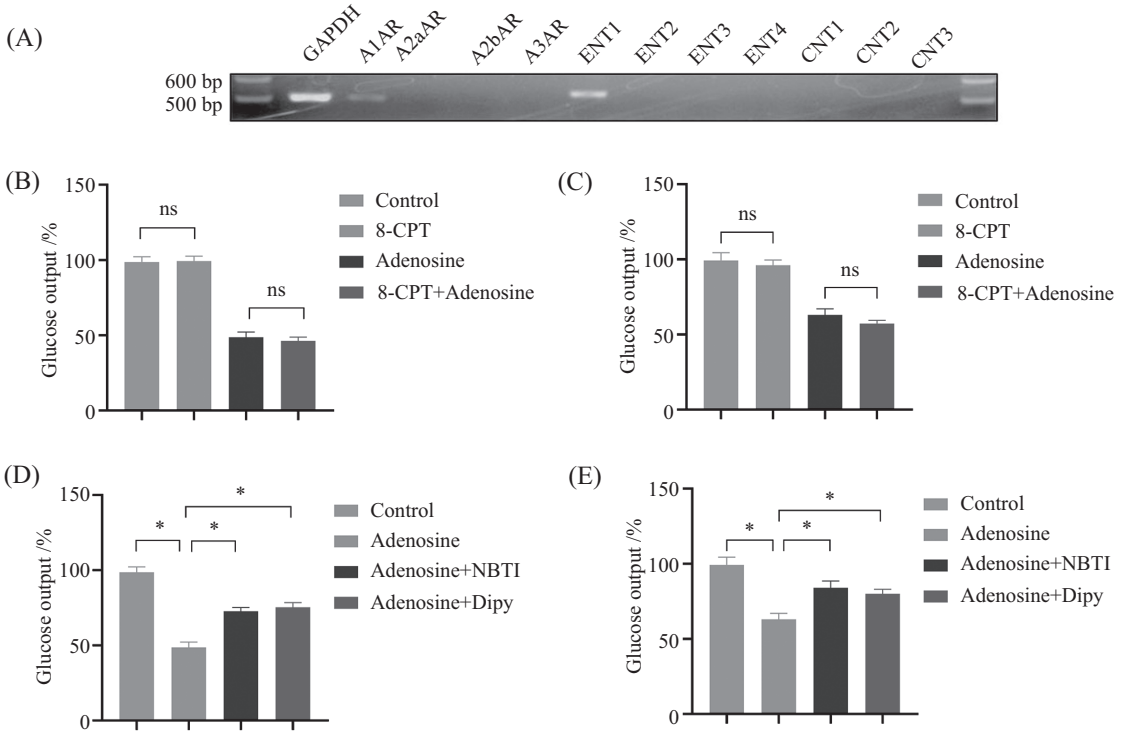
2.5 ENT1介导腺苷对胰高血糖素的调控作用

为明确ENT1在腺苷在胰高血糖素刺激的血糖增高中的功能，我们这里采用了小鼠原代肝细胞和人胚肾细胞HEK293T。

结果显示，胰高血糖素刺激可迅速增加小鼠原代肝细胞的葡萄糖输出，而腺苷可以以剂量依赖的方式显著抑制胰高血糖素诱导的高血糖效应(P<0.05)(图3A)。NBTI或Dipy预处理能够显著逆转腺苷对肝糖输出的抑制作用(P<0.05)(图3B)。在HEK293T细胞中得到类似结果(图3C和图3D)。以上结果证实，腺苷通过ENT1依赖的途径抑制胰高血糖素诱导的肝糖输出。

2.6 腺苷下游的AMPK通路不参与腺苷抑制糖异生的调节作用

细胞内腺苷可通过腺苷激酶(adenosine kinase, AK)转化为单磷酸腺苷(adenosine monophosphate, AMP)，进而激活AMP依赖的蛋白激酶[adenosine 5'-monophosphate (AMP)-activated protein kinase, AMPK]参与细胞能量平衡。为探究腺苷是否通过

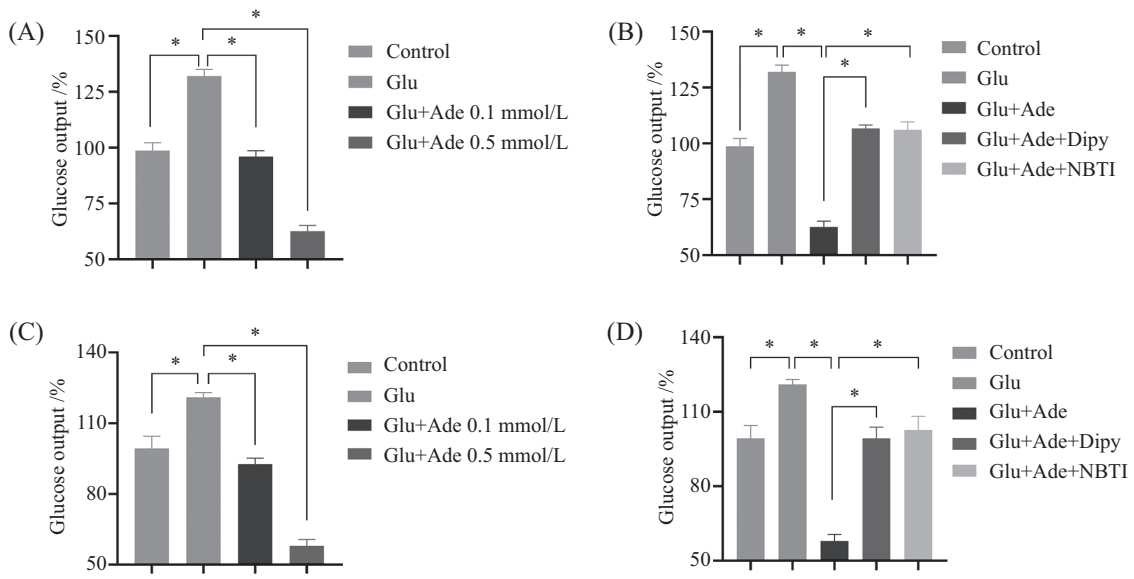


A: RT-PCR定量腺苷受体和核苷转运体在小鼠原代肝细胞中的表达; B: 以8-CPT(20 μmol/L)预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育6 h后检测小鼠原代肝细胞葡萄糖输出(n=3); C: 以8-CPT(20 μmol/L)预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育6 h后检测HEK293T细胞葡萄糖输出(n=3); D: 以Dipy(10 μmol/L)或NBTI(10 μmol/L)分别预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育6 h后检测小鼠原代肝细胞葡萄糖输出(n=3); E: 以Dipy(10 μmol/L)或NBTI(10 μmol/L)分别预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育6 h后检测HEK293T细胞葡萄糖输出(n=3); 8-CPT: 特异性腺苷A1受体抑制剂; Dipy: 非选择性ENT抑制剂; NBTI: 特异性ENT1抑制剂; ^{ns}P>0.05, *P<0.05。

A: RT-PCR quantification of adenosine receptors and nucleoside transporters expression in mouse primary hepatocytes; B: glucose output of mouse primary hepatocytes was measured by pretreating cells with 8-CPT (20 μmol/L) for 1 h and then adding adenosine (0.5 mmol/L) to continue incubation for 6 h (n=3); C: glucose output of HEK293T cells was measured by pretreating cells with 8-CPT (20 μmol/L) for 1 h and then adding adenosine (0.5 mmol/L) to continue incubation for 6 h (n=3); D: glucose output of mouse primary hepatocytes was detected after pretreating the cells with Dipy (10 μmol/L) or NBTI (10 μmol/L) for 1 h, respectively, and then adding adenosine (0.5 mmol/L) to continue the incubation for 6 h (n=3); E: glucose output of HEK293T cells was detected after pretreating the cells with Dipy (10 μmol/L) or NBTI (10 μmol/L) for 1 h, respectively, and then adding adenosine (0.5 mmol/L) to continue the incubation for 6 h (n=3); 8-CPT: selective adenosine A1 receptor antagonist; Dipy: non-selective ENT inhibitor; NBTI: specific ENT1 inhibitor; ^{ns}P>0.05, *P<0.05.

图2 腺苷通过ENT相关途径发挥降血糖作用

Fig.2 Adenosine took hypoglycemic role through ENT related pathway



A: 给予腺苷(0.1或0.5 mmol/L)孵育5.5 h, 实验组添加胰高血糖素(100 nmol/L)孵育0.5 h后检测小鼠原代肝细胞葡萄糖输出(n=3); B: 以Dipy(10 μmol/L)或NBTI(10 μmol/L)分别预处理细胞1 h后加入腺苷(0.5 mmol/L)继续孵育5.5 h, 加入胰高血糖素孵育(100 nmol/L) 0.5 h检测小鼠原代肝细胞葡萄糖输出(n=3); C: 给予腺苷(0.1或0.5 mmol/L)孵育5.5 h后, 实验组添加胰高血糖素(100 nmol/L)孵育0.5 h并检测HEK293T细胞葡萄糖输出(n=3); D: 以Dipy(10 μmol/L)或NBTI(10 μmol/L)分别预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育5.5 h后, 加入胰高血糖素(100 nmol/L)孵育0.5 h检测HEK293T细胞葡萄糖输出(n=3); Glu: 胰高血糖素; Ade: 腺苷; *P<0.05。

A: after administration of adenosine (0.1 or 0.5 mmol/L) for 5.5 h of incubation, glucose output of primary mouse hepatocytes was measured in the experimental group by adding glucagon (100 nmol/L) for 0.5 h of incubation (n=3); B: detection of glucose output of mouse primary hepatocytes. Pretreated the cells with Dipy (10 μmol/L) or NBTI (10 μmol/L) for 1 h, then incubated with adenosine (0.5 mmol/L) for 5.5 h, followed by incubation with glucagon (100 nmol/L) for 0.5 h (n=3); C: after administration of adenosine (0.1 or 0.5 mmol/L) for 5.5 h of incubation, glucose output of HEK293T cells was measured in the experimental group by adding glucagon (100 nmol/L) for 0.5 h of incubation (n=3); D: detection of glucose output of HEK 293T cells. Pretreated the cells with Dipy (10 μmol/L) or NBTI (10 μmol/L) for 1 h, then incubated with adenosine (0.5 mmol/L) for 5.5 h, followed by incubation with glucagon (100 nmol/L) for 0.5 h (n=3); Glu: glucagon; Ade: adenosine; *P<0.05.

图3 腺苷通过ENT相关途径调控胰高血糖素信号转导

Fig.3 Adenosine regulated glucagon signaling through ENT related pathways

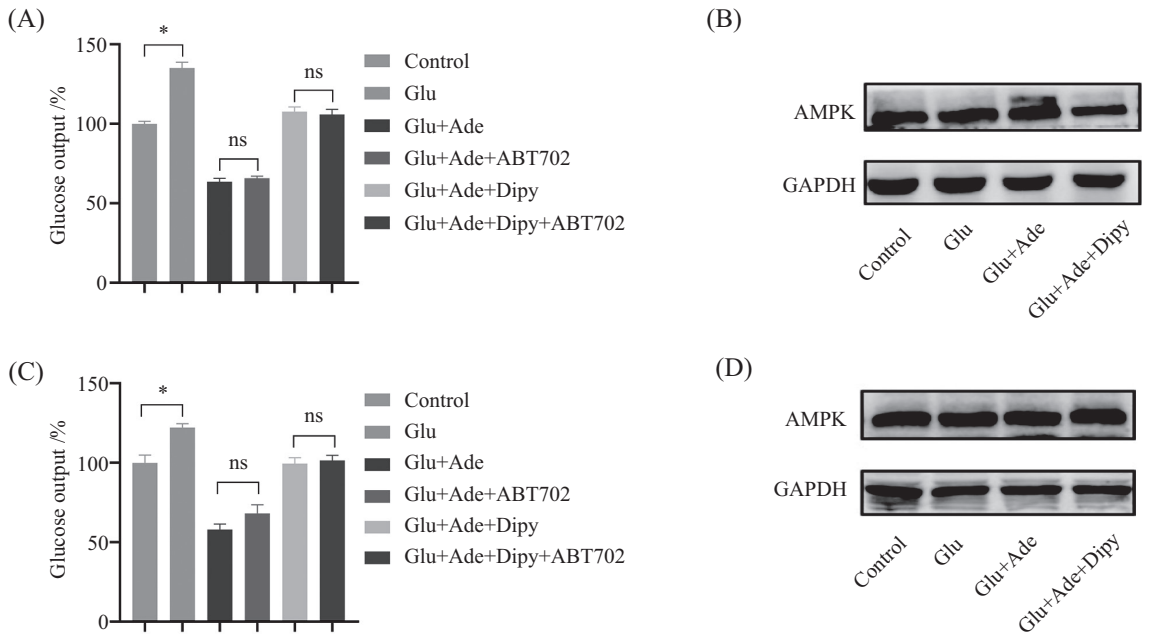
AMPK信号调控糖异生, 我们将AK抑制剂ABT702与胰高血糖素和腺苷三者联合处理小鼠原代肝细胞。有趣的是, ABT702单独使用或与Dipy联用并未影响肝细胞葡萄糖输出(图4A)。同样, 腺苷单独处理或和Dipy联用均不影响原代肝细胞中AMPK蛋白的表达(图4B)。同样地, 在HEK293T细胞中得到类似的验证(图4C和图4D)。这些研究结果提示, 腺苷介导的糖异生抑制并非依赖于AMPK通路的激活。

2.7 cAMP/PKA通路在腺苷诱导的糖异生抑制中的作用

胰高血糖素通过cAMP/PKA信号调节肝脏糖异生关键酶葡萄糖-6-磷酸酶(glucose-6-phosphatase, G-6-pase)和磷酸烯醇式丙酮酸羧激酶(phosphoenolpyruvate carboxykinase, PEPCK)的表达[16]。近期的研究相继报道cAMP/PKA在肥胖和糖尿病中发挥重要作用[17-18]。我们进一步研究腺苷是否通过抑制cAMP/PKA信号来抑制肝脏糖异生。在葡萄糖和腺苷存在下, 我们在小鼠原代肝细胞培养过程中同时加入AC激动剂Forskolin和AC拮抗剂SQ22.136, 并检测细胞的葡萄糖输出水平。结果显示, Forskolin显著抵抗腺苷对葡萄糖诱导的肝脏糖异生的抑制作用(P<0.05), 而SQ22.136进一步增强了这种抑制作用(P<0.05)(图5A)。同时我们利用酶联免疫吸附实验和免疫印迹实验检测了小鼠原代肝细胞下游cAMP和p-PKA的表达。结果显示, 葡萄糖诱导的细胞内cAMP和p-PKA水平的增加受到细胞外腺苷的抑制, 这种效应可以部分被Dipy缓解(图5B和图5C)。在HEK293T细胞中同样检测到相似的表达模式(图5D~图5F)。以上结果表明, 细胞外腺苷通过ENT1抑制AC活性, 进而阻断了cAMP/p-PKA信号, 从而抑制了葡萄糖诱导的糖异生。

3 讨论

肝脏糖异生主要受到激素调控, 而胰高血糖素



A: 以Dipy(10 μmol/L)或ABT702(10 μmol/L)单独或联合预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育5.5 h后, 加入胰高血糖素(100 nmol/L)孵育0.5 h检测小鼠原代肝细胞的葡萄糖输出(n=3); B: Western blot检测小鼠肝原代细胞相关蛋白表达条带图; C: 以Dipy(10 μmol/L)或ABT702(10 μmol/L)单独或联合预处理细胞1 h并加入腺苷(0.5 mmol/L)继续孵育5.5 h后, 加入胰高血糖素(100 nmol/L)孵育0.5 h检测HEK293T细胞的葡萄糖输出(n=3); D: Western blot检测HEK293T细胞相关蛋白表达条带图; Glu: 胰高血糖素; Ade: 腺苷; ^{ns}P>0.05, *P<0.05。

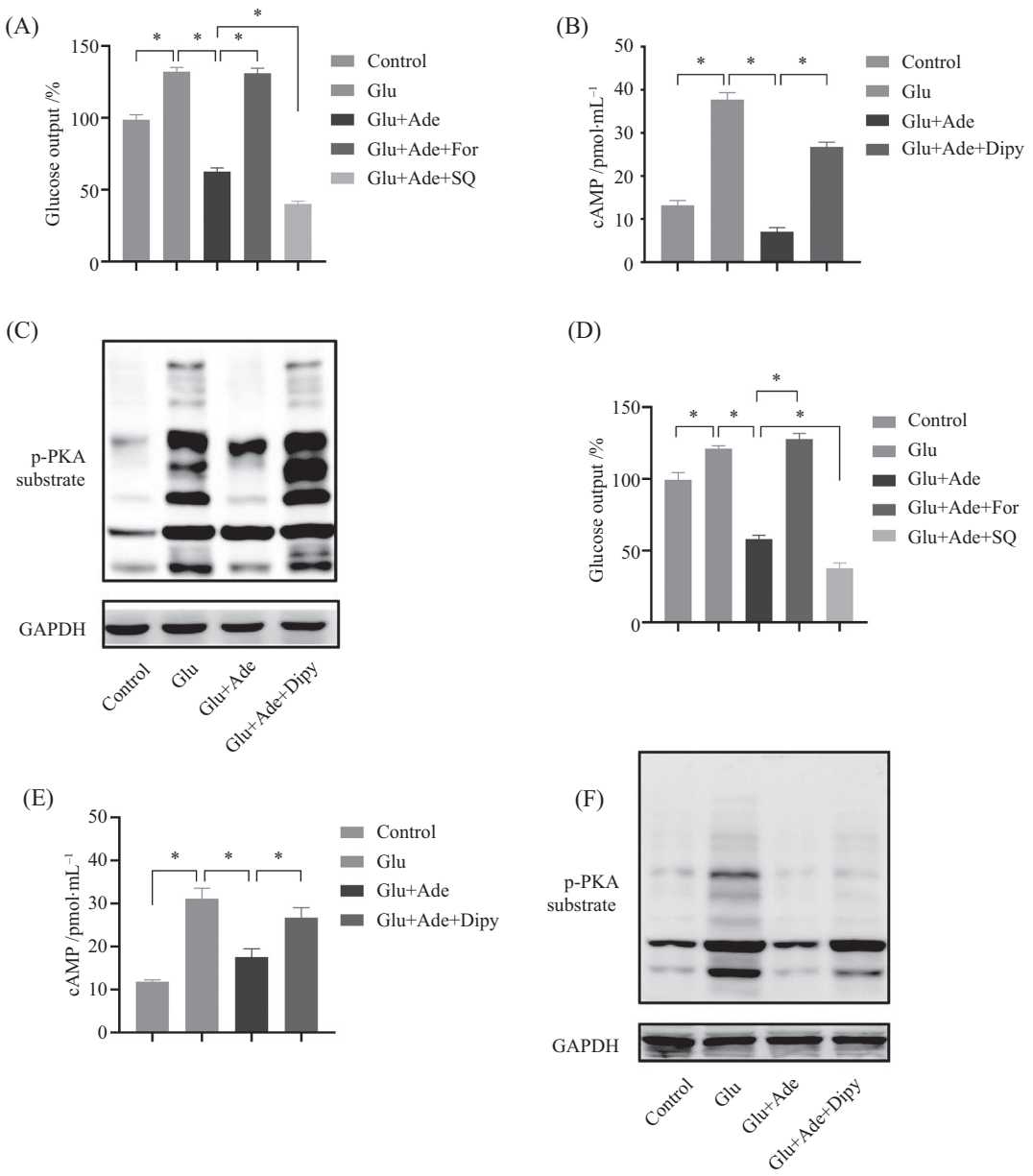
A: primary mouse liver cells were pretreated with Dipy (10 μmol/L) or ABT702 (10 μmol/L) alone or in combination for 1 h, then incubated with adenosine (0.5 mmol/L) for 5.5 h followed by treatment with glucagon (100 nmol/L) for 0.5h. Then the glucose output of primary mouse liver cells was detected (n=3); B: Western blot detection of protein expression in primary mouse liver cells and HEK293T cells; ABT702: adenosine kinase inhibitor. Glucose output of HEK293T cells; C: HEK293T cells were pretreated with Dipy (10 μmol/L) or ABT702 (10 μmol/L) alone or in combination for 1 h, then incubated with adenosine (0.5 mmol/L) for 5.5 h followed by treatment with glucagon (100 nmol/L) for 0.5 h. Then the glucose output of HEK293T cells was detected (n=3); D: Western blot detection of mouse liver progenitor cells and HEK293T cells related protein expression strip maps; Glu: glucagon; Ade: adenosine; ^{ns}P>0.05, *P<0.05.

图4 腺苷通过独立于AMPK的途径抑制肝脏葡萄糖生成

Fig.4 Adenosine suppressed liver gluconeogenesis via a pathway independent of AMPK

是其最重要的促进剂。大量研究从不同角度解析了胰高血糖素介导的肝糖异生途径。研究表明, 胰高血糖素与细胞表面Gαs受体结合, 活化cAMP/PKA信号, 进而诱导cAMP反应元件结合蛋白(cyclic-AMP response binding protein, CREB)、IP3R等蛋白磷酸化, 上调糖异生基因表达[19]。然而, 胰高血糖素诱导cAMP/PKA信号通路活化的上游调控机制仍有待深入研究。本研究通过经典的糖异生评估实验(丙酮酸耐受和胰高血糖素刺激实验)发现, 腺苷显著抑制糖异生引起的血糖升高。在小鼠原代肝细胞和HEK293T细胞中, 细胞外腺苷在短时间内即可迅速抑制胰高血糖素诱导的cAMP增加, 并抑制PKA及其下游蛋白磷酸化, 减少细胞糖输出。因此, 我们的研究结果揭示了细胞外腺苷在糖异生调节网络中起着关键作用, 是T2D的潜在治疗药物。

作为一种重要的内源性信号分子和临床用药"多面手", 腺苷能够诱导血管扩张, 调节交感神经系统的活动, 具有抗血栓形成的特性, 并能降低血压和心率, 在临床上(如治疗室上性心动过速、脑血管障碍、原发性高血压等)得到广泛应用[20-22]。此外, 腺苷及其受体在糖脂代谢及糖尿病发生发展中的作用也得到了广泛挖掘。研究发现, 腺苷通过其膜受体及转运体调节胰岛素分泌、葡萄糖释放和清除、糖原分解和糖生成等过程[23], 维持葡萄糖稳态。在骨骼肌中, 腺苷刺激A1AR可促进糖摄入, 而A2bAR激活则引起糖摄入减少[24-25]。此外, ARs的抗炎作用能够保护胰腺β细胞, 提高β细胞活力[26]。然而, 腺苷对肝脏内糖异生调节及其作用机制未有定论。本研究发现了外源性腺苷能够调控肝脏糖异生通路的改变。在体内模型中, 外源性腺苷显著抑制小鼠血糖升高。在细胞模型中, 腺苷以剂量依赖的方式抑制肝脏糖异生进而降低葡萄糖输出水平, 且无



A: 给予腺苷(0.5 mmol/L)联合Forskolin(10 μmol/L)或SQ22.136(10 μmol/L)处理5.5 h后, 加入胰高血糖素(100 nmol/L)孵育0.5 h并检测小鼠原代肝细胞的葡萄糖输出(n=3); B: ELISA法测定小鼠原代肝细胞培养上清液中的cAMP浓度。以Dipy(10 μmol/L)预处理细胞1 h后加入腺苷(0.5 mmol/L)继续孵育5.5 h, 加入胰高血糖素孵育(100 nmol/L) 0.5 h(n=3); C: Western blot检测小鼠肝原代细胞相关蛋白表达条带图; D: 给予腺苷(0.5 mmol/L)联合Forskolin(10 μmol/L)或SQ22.136(10 μmol/L)处理5.5 h后, 加入胰高血糖素(100 nmol/L)孵育0.5 h并检测HEK293T细胞的葡萄糖输出(n=3); E: ELISA法测定HEK293T细胞培养上清液中的cAMP浓度。以Dipy(10 μmol/L)预处理细胞1 h后加入腺苷(0.5 mmol/L)继续孵育5.5 h, 加入胰高血糖素孵育(100 nmol/L) 0.5 h(n=3); F: Western blot检测HEK293T细胞相关蛋白表达条带图; Glu: 胰高血糖素; Ade: 腺苷; For: 佛司可林; SQ: SQ22.136; *P<0.05。

A: glucose output of mouse primary hepatocytes was detected after administration of adenosine (0.5 mmol/L) combined with Forskolin (10 μmol/L) or SQ22.136 (10 μmol/L) treatment for 5.5 h, glucagon (100 nmol/L) was added and incubated for 0.5 h (n=3); B: cAMP concentration in the supernatant of mouse primary hepatocyte culture was determined by ELISA. Cells were pretreated with Dipy (10 μmol/L) for 1 h and then adenosine (0.5 mmol/L) was added to continue the incubation for 5.5 h. After that, glucagon was added to incubate (100 nmol/L) for 0.5 h (n=3); C: Western blot detection of mouse primary liver cell-associated protein expression banding graphs; D: Glucose output of HEK 293T cells was detected after administration of adenosine (0.5 mmol/L) combined with Forskolin (10 μmol/L) or SQ22.136 (10 μmol/L) treatment for 5.5 h, glucagon (100 nmol/L) was added and incubated for 0.5 h (n=3); E: ELISA to determine the cAMP concentration in HEK293T cell culture supernatant. Cells were pretreated with Dipy (10 μmol/L) for 1 h and then adenosine (0.5 mmol/L) was added to continue the incubation for 5.5 h. After that, glucagon was added to incubate (100 nmol/L) for 0.5 h (n=3); F: Western blot detection of HEK293T cell-associated protein expression banding graph; Glu: glucagon; Ade: adenosine; For: forskolin; SQ: SQ22.136; *P<0.05.

图5 腺苷通过ENT相关途径抑制腺苷酸环化酶活性

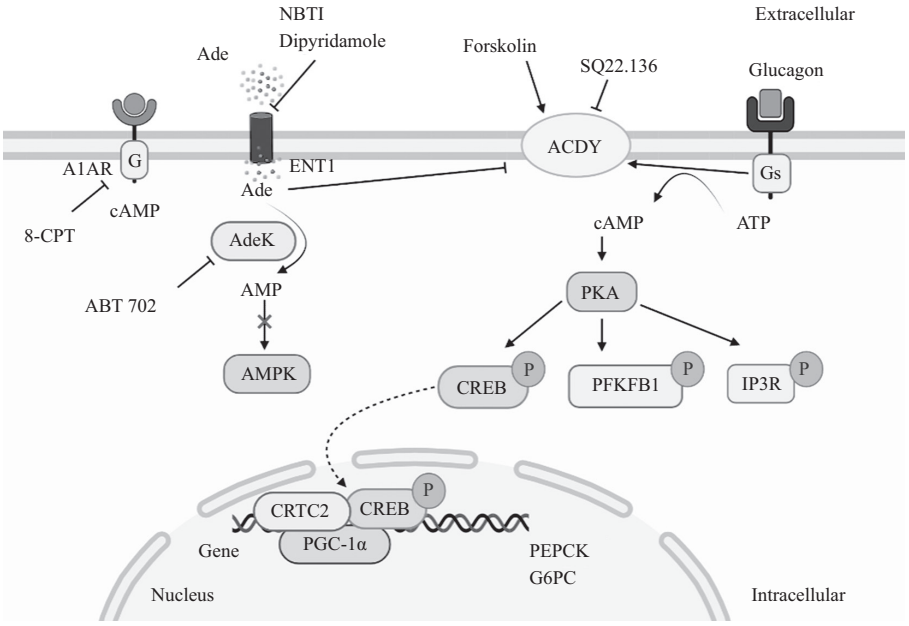
Fig.5 Adenosine inhibited adenylyl cyclase activity via equilibrative nucleoside transporter related pathways

细胞毒性。

ENTs主要包括1~4四种亚型。其中, ENT1广泛分布于所有细胞和组织中, 是最主要的核苷转运体[27]。既往关于转运体蛋白ENT的研究集中于神经细胞和心肌细胞中对腺苷的双向转运[28], 而在糖脂代谢器官中的报道鲜见。有研究发现, 敲除*ENT1*可导致肾纤维化和肾上皮细胞的上皮−间质转化, 诱发糖尿病肾病[29]。SUAREZ等[30]发现, 胰岛素对ENT2活性的调节不足与糖尿病肾小球病变密切相关。最近的一项研究揭示, ENT1参与了脂肪细胞产热程序激活和白色脂肪“棕色化”, 而人类ENT1的突变与BMI明显降低有关[31]。在本研究中, RT-PCR结果显示, 肝脏作为糖异生最主要的器官主要表达核苷转运体ENT1。联合运用ENT抑制剂后, 腺苷抑制糖异生的作用显著减弱, 这个现象提示腺苷对糖异生的调节作用可能依赖于核苷转运体而非传统的腺苷受体。上述结果表明, ENT可能在肝脏糖异生的嘌呤能信号调节中扮演关键角色, 可能为腺苷的新药研发提供新方向。

在肝脏中, 胰高血糖素激活了下游的AMPK和cAMP/PKA信号通路, 诱导糖异生[32-33]。越来越多的研究表明, cAMP/PKA信号通路在调节多个系统的能量平衡和糖脂代谢方面具有核心作用, 如在棕色脂肪细胞中, 冷刺激等激活cAMP/PKA通路, 进一步诱导脂肪产热增加[34]。抑制PKA能够延缓结肠癌小鼠的脂肪萎缩[35]。在人类视网膜色素上皮细胞, 激活cAMP/PKA信号通路, 可以改善高葡萄糖诱导的细胞炎症和凋亡[36]。据报道, AK抑制剂ABT702可以抑制细胞内腺苷的再循环和AMP的产生, 进一步调节AMPK下游信号转导[37]。有趣的是, 本研究发现联合应用非选择性ENT抑制剂Dipy和特异性ENT1抑制剂ABT 702后, 细胞的糖异生抑制并未受到影响, 进一步提示转运进入细胞内的腺苷直接影响了AC活性, 调控了糖异生。膜结合型AC可在胰高血糖素刺激下活化, 诱导第二信使cAMP的产生增加, 进一步磷酸化下游靶点PKA, 最终激活肝脏糖异生[38]。GNAD等[39]发现, 在脂肪细胞中, 胞外腺苷可能通过不同受体激活或抑制AC, 调节cAMP/PKA信号。我们在细胞模型中发现, 腺苷显著降低细胞内的cAMP水平, 并抑制PKA的磷酸化。本研究后续将进一步详细探究细胞外腺苷如何影响AC的机制, 以及下游糖异生转录因子的调控。

综上所述, 细胞外腺苷通过转运蛋白ENT1进入细胞, 抑制AC活性, 通过阻断cAMP积累和PKA磷酸化, 抑制肝脏糖异生, 最终减少糖输出(图6)。本研



外源性腺苷通过转运蛋白ENT1入胞内, 抑制胰高血糖素/cAMP/PKA信号通路, 进而减少肝糖输出。

Extracellular adenosine entered the cell via the adenosine transporter ENT1 and inhibited the glucagon/cAMP/PKA signaling pathway, resulting in hepatic glucose output reduction.

图6 腺苷通过调控胰高血糖素/cAMP/PKA信号通路抑制葡萄糖生成的模式图

Fig.6 Schematic of adenosine inhibition on gluconeogenesis via glucagon/cAMP/PKA signaling pathway

究初步揭示了细胞外腺苷调控体内外糖异生模型的新机制，为腺苷治疗伴有糖异生异常型糖尿病提供科学支撑。本研究后续将继续开展腺苷调控肝脏糖异生的分子机制的积极探索，在糖尿病动物模型中进一步验证腺苷对糖尿病潜在的治疗作用及cAMP/p-PKA信号在其中的效应及下游机理。

参考文献 (References)

[1] COLLABORATORS G B D D. Global, regional, and national burden of diabetes from 1990 to 2021, with projections of prevalence to 2050: a systematic analysis for the Global Burden of Disease Study 2021 [J]. Lancet, 2023, 402(10397): 203-34.

[2] SUN H, SAEEDI P, KARURANGA S, et al. IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045 [J]. Diabetes Res Clin Pract, 2022, 183: 109119.

[3] RODEN M, SHULMAN G I. The integrative biology of type 2 diabetes [J]. Nature, 2019, 576(7785): 51-60.

[4] BODEN G. Gluconeogenesis and glycogenolysis in health and diabetes [J]. J Investig Med, 2004, 52(6): 375-8.

[5] SHAH A, WONDISFORD F E. Gluconeogenesis flux in metabolic disease [J]. Annu Rev Nutr, 2023, 43: 153-77.

[6] PETERSEN M C, VATNER D F, SHULMAN G I. Regulation of hepatic glucose metabolism in health and disease [J]. Nat Rev Endocrinol, 2017, 13(10): 572-87.

[7] ALLARD B, LONGHI M S, ROBSON S C, et al. The ectonucleotidases CD39 and CD73: novel checkpoint inhibitor targets [J]. Immunol Rev, 2017, 276(1): 121-44.

[8] RIVKEES S A, WENDLER C C. Long-term consequences of disrupting adenosine signaling during embryonic development [J]. Mol Aspects Med, 2017, 55: 110-7.

[9] CHIEN T, WENG Y T, CHANG S Y, et al. GSK3β negatively regulates TRAX, a scaffold protein implicated in mental disorders, for NHEJ-mediated DNA repair in neurons [J]. Mol Psychiatry, 2018, 23(12): 2375-90.

[10] PFAFFENZELLER M S, FRANCIOSI M L M, CARDOSO A M. Purinergic signaling and tumor microenvironment in cervical Cancer [J]. Purinergic Signal, 2020, 16(1): 123-35.

[11] GRANADE M E, HARGETT S R, LANK D S, et al. Feeding desensitizes A1 adenosine receptors in adipose through FOXO1-m ediated transcriptional regulation [J]. Mol Metab, 63: 101543.

[12] ZHANG J, HOU Y, DU X L, et al. $ADORA_1$-driven brain-sympathetic neuro-adipose connections c ontrol body weight and adipose lipid metabolism [J]. Mol Psychiatry, 26(7): 2805-19.

[13] KROPOTOV A, KULIKOVA V, NERINOVSKI K, et al. Equilibrative nucleoside transporters mediate the import of nicotinamide riboside and nicotinic acid riboside into human cells [J]. Int J Mol Sci, 2021, 22(3): 1391.

[14] LIU Q, ZHANG F G, ZHANG W S, et al. Ginsenoside Rg1 inhibits glucagon-induced hepatic gluconeogenesis through Akt-FoxO1 interaction [J]. Theranostics, 2017, 7(16): 4001-12.

[15] BOISON D, YEGUTKIN G G. Adenosine metabolism: emerging concepts for cancer therapy [J]. Cancer Cell, 2019, 36(6): 582-96.

[16] ALTAREJOS J Y, MONTMINY M. CREB and the CRTC co-activators: sensors for hormonal and metabolic signals [J]. Nat Rev Mol Cell Biol, 2011, 12(3): 141-51.

[17] CHEN Y, WANG J, WANG Y, et al. A propolis-derived small molecule ameliorates metabolic syndrome in obese mice by targeting the CREB/CRTC2 transcriptional complex [J]. Nat Commun, 2022, 13(1): 246.

[18] WANG Y, LIU Q, KANG S G, et al. Dietary bioactive ingredients modulating the cAMP signaling in diabetes treatment [J]. Nutrients, 2021, 13(9): 3038.

[19] YANG M, PAN M, HUANG D, et al. Glucagon promotes gluconeogenesis through the GCGR/PKA/CREB/PGC-1α pathway in hepatocytes of the Japanese flounder *Paralichthys olivaceus* [J]. Cells, 12(7): 1098.

[20] ZHANG Y, WERNLY B, CAO X, et al. Adenosine and adenosine receptor-mediated action in coronary microcirc ulation [J]. Basic Res Cardiol, 116(1): 22.

[21] LAYLAND J, CARRICK D, LEE M, et al. Adenosine: physiology, pharmacology, and clinical applications [J]. JACC Cardiovasc Interv, 7(6): 581-91.

[22] ANTONIOLI L, FORNAI M, PELLEGRINI C, et al. Adenosine signaling as target in cardiovascular pharmacology [J]. Curr Opin Pharmacol, 2023, 71: 102393.

[23] SUBIABRE M, VILLALOBOS-LABRA R, SILVA L, et al. Role of insulin, adenosine, and adipokine receptors in the foetoplacen tal vascular dysfunction in gestational diabetes mellitus [J]. Biochim Biophys Acta Mol Basis Dis, 2020, 1866(2): 165370.

[24] LI Y, LUO S, MA R, et al. Upregulation of cytosolic phosphoenolpyruvate carboxykinase is a critical metabolic event in melanoma cells that repopulate tumors [J]. Cancer Res, 2015, 75(7): 1191-6.

[25] SANDERS E, DIEHL S. Analysis and interpretation of transcriptomic data obtained from extended Warburg effect genes in patients with clear cell renal cell carcinoma [J]. Oncoscience, 2015, 2(2): 151-86.

[26] VETERE A, CHOUDHARY A, BURNS S M, et al. Targeting the pancreatic beta-cell to treat diabetes [J]. Nat Rev Drug Discov, 2014, 13(4): 278-89.

[27] PASTOR-ANGLADA M, PÉREZ-TORRAS S. Who is who in adenosine transport [J]. Front Pharmacol, 2018, 9: 627.

[28] RUAN W, LI J, CHOI S, et al. Targeting myocardial equilibrative nucleoside transporter ENT1 provide s cardioprotection by enhancing myeloid Adora2b signaling [J]. JCI Insight, 8(11): e166011.

[29] GUILLÉN-GÓMEZ E, PINILLA-MACUA I, PÉREZ-TORRAS S, et al. New role of the human equilibrative nucleoside transporter 1 (hENT1) in epithelial-to-mesenchymal transition in renal tubular cells [J]. J Cell Physiol, 2012, 227(4): 1521-8.

[30] SUAREZ R, VILLARREAL C, NAHUELPÁN Y, et al. Defective insulin-stimulated equilibrative nucleoside transporter-2 ac tivity and altered subcellular transporter distribution drive the loss of adenosine homeostasis in diabetic kidney disease progression [J]. Biochim Biophys Acta Mol Basis Dis, 2024, 1870(1): 166890.

[31] NIEMANN B, HAUFS-BRUSBERG S, PUETZ L, et al. Apoptotic brown adipocytes enhance energy expenditure via extracellular inosine [J]. Nature, 2022, 609(7926): 361-8.

[32] BENCHOULA K, PARHAR I S, MADHAVAN P, et al. CREB

nuclear transcription activity as a targeting factor in the treatment of diabetes and diabetes complications [J]. Biochem Pharmacol, 2021, 188: 114531.

[33] YU X, MENG Z, FANG T, et al. Empagliflozin inhibits hepatic gluconeogenesis and increases glycogen synthesis by AMPK/CREB/GSK3β signalling pathway [J]. Front Physiol, 2022, 13: 817542.

[34] CHOI K M, CHO S H, KIM J H, et al. CFTR regulates brown adipocyte thermogenesis via the cAMP/PKA signalin g pathway [J]. J Cyst Fibros, 22(1): 132-9.

[35] WANG R, WEI L, WAZIR J, et al. Curcumin treatment suppresses cachexia-associated adipose wasting in m ice by blocking the cAMP/PKA/CREB signaling pathway [J]. Phytomedicine, 2023, 109: 154563.

[36] ZHANG Y, MENG X, LIU K. The modulation of cAMP/PKA pathway by asiaticoside ameliorates high glucose-induced inflammation and apoptosis of retinal pigment epithelial cells [J]. J Bioenerg Biomembr, 2022, 54(1): 9-16.

[37] CHOI K Y, AHN Y H, AHN H W, et al. Involvement of Akt2/protein kinase Bβ (PKBβ) in the 8-Cl-cAMP-induced cancer cell growth inhibition [J]. J Cell Physiol, 2013, 228(4): 890-902.

[38] STOZER A, PARADIZ LEITGEB E, POHOREC V, et al. The role of cAMP in β cell stimulus-secretion and intercellular coupling [J]. Cells, 2021, 10(7): 1658.

[39] GNAD T, SCHEIBLER S, VON KUGELGEN I, et al. Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors [J]. Nature, 2014, 516(7531): 395-9.