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EFFECTS OF INSULIN ON ADAPTIVE CAPACITY OF RAT PANCREATIC ACINAR CELLS MITOCHONDRIA

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Insulin increases the basal and agonist-stimulated secretion of pancreatic acinar cells, which leads to increase of energy demand and requires sufficient oxidative substrates supply. Cholecystokinin substantially increases the respiration rate of pancreatic acinar cells upon pyruvate oxidation. However, it is not clear how insulin affects mitochondrial oxidative processes at rest and upon secretory stimulation. Experiments were carried out on male Wistar rats (250–300 g) kept on standard diet. Animals were fasted 12 h before the experiment. Pancreatic acini were isolated with collagenase. Basal and FCCP-stimulated respiration of rat pancreatic acini was measured with Clark electrode. Adaptive capacity of mitochondria was assessed by the maximal rate of uncoupled respiration. Statistical significance (P) of differenced between the means was assessed either with a paired t-test or with repeated measures two-way ANOVA and post-hoc Turkey test. Adaptive capacity of pancreatic acinar mitochondria was significantly higher when pyruvate (2 mM) was used as oxidative substrate comparing with glucose (10 mM). Incubation with insulin (100 nM) for 20 minutes elevated the basal respiration and adaptive capacity of pancreatic acinar mitochondria upon glucose, but not pyruvate, oxidation. Cholecystokinin (0.1 nM, 30 min) stimulated the rate of basal and maximal uncoupled respiration of acinar cells upon pyruvate oxidation, but insulin completely negated this increase of mitochondrial adaptive capacity. Thus, insulin increases the glucose oxidation in pancreatic acinar cells at resting state, but suppresses pyruvate oxidation upon secretory stimulation with cholecystokinin. The mechanisms of insulin action of pyruvate metabolism in pancreatic acinar cells require further elucidation.

Keywords: pancreatic acini, insulin, cholecystokinin, glucose, pyruvate

Introduction

Exocrine panceas secretion is regulated by neuromediator acetylcholine and gastrointestinal hormones, such as secretin and cholecystokinin. The role of insulin in maintaining normal functioning of pancreatic acinar cells is also important. Interaction between endocrine and exocrine pancreas is very active due to close localization and direct bloodstream connection. Thus, high concentrations of insulin secreted by β-cells primarily reach acinar cells [24] affecting basal and stimulated secretion [7, 20, 21], gene expression and synthesys of digestive enzymes [22, 11]. It is well-known that diabetes mellitus is frequently accompanied by exocrine pancreatic insufficiency [8].

Pancreatic acinar cells secretory function requires high level of mitochondrial ATP production [23, 14, 1]. Insulin effects on mitochondrial oxidative processes in pancreatic acinar cells were not studied yet. A reliable approach to detect changes of mitochondrial fucntions in live cells is evaluation of adaptive respiratory response to protonophore [15]. Thus, the aim of present work was to investigate the effects of insulin on respiration of pancreatic acinar cells in rest and upon secretory stimulation with cholecystokinin.

Materials and Methods

All manipulations with animals are accomplished in accordance with 'European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes' (Council of Europe No 123, Strasbourg, 1985). Experimental protocols were approved by the Animal Care and Use Committee of Ivan Franko National University of Lviv. Experiments were carried out on male Wistar rats (250–300 g). Animals were kept under the standard conditions of vivarium at the constant temperature with 12:12-h light-dark cycle and on the basic diet. Animals were fasted 12 h before the experiment.

A suspension of isolated pancreatic acini was obtained with collagenase (type IV, 220 U/ml) as previously described [14]. Cell calculation was performed with a haemocytometer. Cell viability after isolation was >93 % as assessed with trypan blue test.

The rate of oxygen consumption was measured with the Clark oxygen electrode (Biological oxygen monitor YSI 5300, USA) in the closed 1.6-mL glass respiration chamber at 37 °C. Respiration rate was calculated assuming 1 ml of solution contained 200 nmoles O_2.

Isolated pancreatic acini were obtained and stored in isolation medium, containing, mM: NaCl – 140.0, KCl – 4.7, $CaCl_2$ – 1.3, $MgCl_2$ – 1.0, HEPES – 10.0, glutamine – 2.0, pyruvate – 2.0, glucose – 10.0; BSA – 2.5 mg/ml; soybean tripsin inhibitor – 0.1 mg/ml and minimum essential amino acid supplement for MEM; pH 7.4. Incubation media were similar to this composition but without glutamine and amino acid supplement. The media also contained either glucose (10 mM) or pyruvate (2 mM). After incubation for 20–30 min 0.6 ml of acini suspension was added into the respiratory chamber containing additional 1 ml of incubation medium. The respiration was stimulated with protonophore FCCP at increasing concentrations (0.5, 1, 1.5 and 2 μM). Maximal rate of uncoupled respiration was not always reached at the same FCCP concentration. Thus it was calculated as a mean of maximal rates of each individual experiment at different "optimal" FCCP concentrations.

All reagenst used in experiments were of high purity and usually manufactured by Sigma-Aldrich. Each experiment was repeated on at least four separate preparations of isolated asini from different animals ($n \geq 4$). Statistical and mathematical calculations were performed using Microsoft Excel. Numerical values are presented as M±S.E.M. Statistical significance (P) of differenced between the means was assessed either with a paired t-test or with repeated measures two-way ANOVA and post-hoc Turkey test.

Results and Discussion

In a first experiment the suspension of isolated pancreatic acini was incubated with insulin (100 nM) for 20 min at 37 °C in basic solution conteining either glucose or pyruvate. The rate of basal respiration did not depend on oxidative substrate (Fig. 1, *A, B*), which is consistent with previous data [14]. Insulin significantly stimulated basal respiration of acini upon glucose presence in medium – by 28 % comparing to control (Fig. 1, *A*). Insulin caused no effect on basal respiration, when pyruvate was used instead glucose (Fig. 1, *B*).

In control, the rate of FCCP-stimulated respiration also depended on oxidative substrate. Upon glucose oxidation, adding 0.5 μM FCCP to the respiration chamber caused an increase of respiration rate to 1.95 ± 0.44 r.u. At higher concentrations, FCCP caused gradual decrease of respiration rate (Fig. 1, *A*). When pyruvate was used as energetic substrate, 0.5, 1 and 1.5 μM FCCP elevated respiration to 1.89 ± 0.16, 2.52 ± 0.24 and 2.56 ± 0.38 r.u., respectively. Only 2 μM FCCP caused a small decline of respiration rate (Fig. 1, *C*).

After incubation with insulin upon glucose oxidation maximal uncoupled respiration (0.5 μM FCCP) significantly increased by 18 % comparing to control (Fig. 1, *A* and *B*). When

pyruvate was used instead, insulin did not change FCCP-stimulated respiration of pancreatic acini (Fig. 1, *C, D*).

Described changes of respirations support the data that insulin in exocrine pancreas, similarly to classic insulin-sensitive tissues, stimulates glucose accumulation and metabolism [10]. In pancreatic acinar cells insulin is known to stimulate glycolytic, but not mitochondrial production of NAD(P)H [13]. Supposedly, these data mean that insulin might switch energetic catabolism of pancreatic acinar cells from mitochondrial to glycolytic [13]. Hovewer, stimulation of basal respiration rate by insulin upon glucose oxidation indicates not olny increased glucose transport, but also increased mitochondrial oxidation. We assume that this increase is due to either utilization of cytosolic NAD(P)H by mitochondria (e.g. via malate-aspartate shuttle), or additional pyruvate supply as a result of glycolizis stimulation.

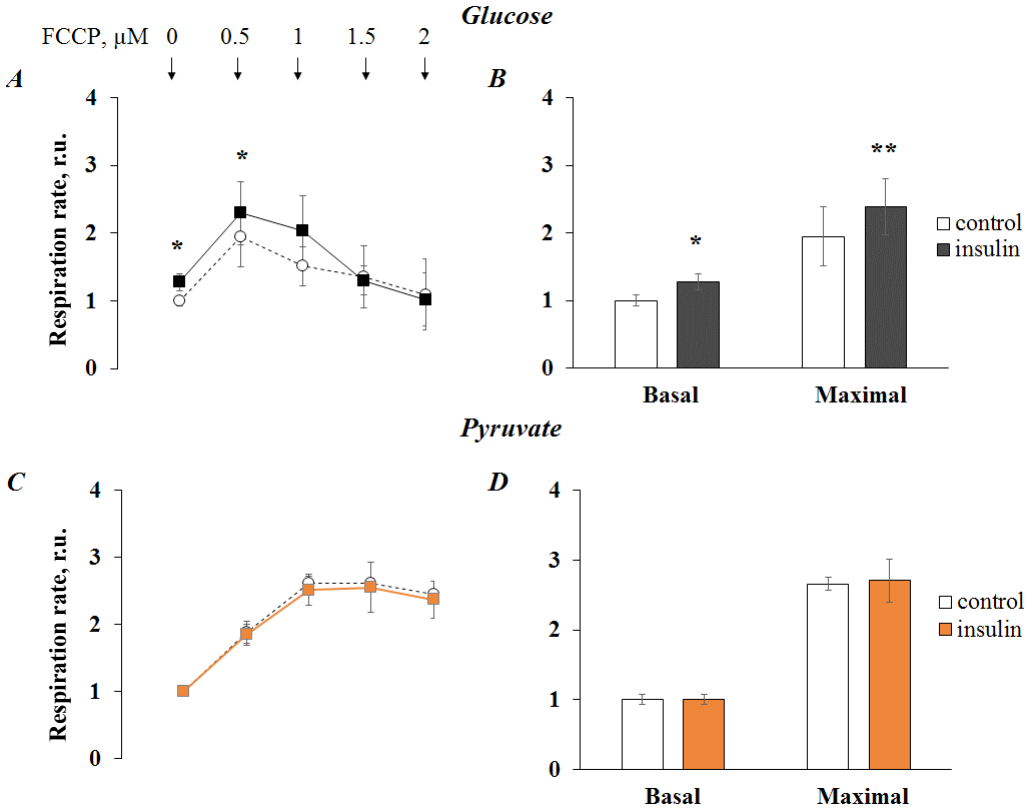

Fig. 1. Insulin effects on respiration of pancreatic acini depends on oxidative substrate: *A, B* – glucose (10 mM); *C, D* – pyruvate (2 mM); [insulin] = 100 nM; maximal respiration (*B, D*) is respiration upon optimal FCCP concentration; data normalized to basal respiration (no FCCP) taken as 1 r.u.; * – significant change of respiration rate comparing to control, paired t-test, $P < 0.05$ ** – $P < 0.01$, $n = 4$, mean ± S.E.M.

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Gastriointestinal hormone cholecystokinin is known to cause the increase of maximal uncoupled respiration of pancreatic acini only upon oxidation of pyruvate [1]. In the second set of experiments, we have tested if insulin modulates the cholecystokinin action on basal and uncoupled respiration. Suspension of isolated acini were incubated for 30 min at 37 °C in pyruvate-supplemented solution with cholecystokinin (0.1 nM) or/and insulin (100 nM). The effects of insulin of respiration were similar to the first experiment. Cholecystokinin expectedly stimulated the rate of basal and maximal uncoupled respiration of acinar cells (by 64 and 35 % respecrively, Fig. 2). Insulin completely abolished the effects of cholecystokinin on respiration (two-way ANOVA, Fig. 2).

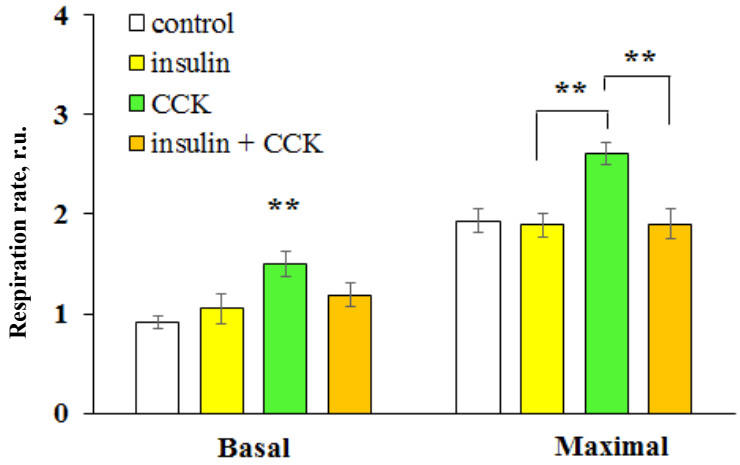

Fig. 2. Insulin inhibits the effect of CCK on basal and FCCP-stimulated respiration of pancreatic acini upon pyruvate oxidation: [CCK] – 0.1 nM, [insulin] – 100 nM, [pyruvate] – 2 mM, [FCCP] – 0.5–2 μM; data normalized to basal respiration (no FCCP) upon glucose oxidation, ** – significant change of respiration rate comparing to control, paired t-test, $P < 0.01$ or between samples, ANOVA, post-hoc Turkey test, $P \leq 0.01$, $n = 6–7$, mean ± S.E.M.

The mechanism of cholecystokinin-stimulated pyruvate oxidation suppression by insulin is not clear. We suppose that any changes of gene expression could not manifest themselves within experimental timeframes (20–30 min). Thus, mechanism of insulin action is associated with modification of present signaling of metabolic proteins.

One hypothesis is that insulin affects signal transduction from cholecystokinin receptors. Currently no data indicate that this is realized via modulation of Ca^{2+}-transporting systems. Insulin is known to affect the activity of plasma membrane Ca^{2+} ATPase only indirectly via the change of cytosolic [ATP] [13]. Othe data prove that insulin may change the affinity and capacity of different cholecystokinin receptors to cholecystokinin in pancreatic acinar cells [17]. This was the primary cause of detected alteration of secretory response to cholecystokinin. Hovewer the data about insulin influence on stimulated secretion are ambiguous. Several authors have shown that exogenous insulin upon physiological glucose concentration does not influence basal protein and fluid secretion, but potentiates secretory stimulation by cholecystokinin and acetylcholine [7, 9, 12, 20, 21]. In other experimnets high glucose level and exogenous insuline inhibited

cholechestokinine-stimulated pancreatic secretion [4]. Still, most of recent studies support the potentiation of basal and stimulated secretion of pancreatic acinar cells by insulin [2, 9, 19]. Thus we suppose that the decrease of pyruvate oxidation could not be a consequence of secretion stimulation by insulin.

Alternative hypothesis is that the detected insulin effect is a result of direct influence on cell metabolism. It is known that insulin causes the decrease of mitochondrial and increase of glycolytic NADH production in pancreatic acinar cells [13]. Still apparently insulin does not directly inhibit the pyruvate dehydrogenase complex in pancreatic acinar cells, because this hormone did not influence the respiration rate upon pyruvate presence without cholecystokinin. Moreover, in other tissues (liver and adipose tissue) insulin activates pyruvate dehydrogenase [18, 5]. Clot et al. [3] have shown that maximal pyruvate dehydrogenase activity in isolated liver cells was achieved after 10 min of insulin action and was maintained at least for 45 min, which is consistent with our experimental settings.

Thus, insulin increases the glucose oxidation in pancreatic acinar cells at resting state, but suppresses pyruvate oxidation upon secretory stimulation with cholecystokinin. The mechanisms of insulin action of pyruvate metabolism in pancreatic acinar cells require further elucidation.

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ВПЛИВ ІНСУЛІНУ НА АДАПТАЦІЙНУ ЗДАТНІСТЬ МІТОХОНДРІЙ АЦИНАРНИХ КЛІТИН ПІДШЛУНКОВОЇ ЗАЛОЗИ ЩУРІВ

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Інсулін збільшує базальну та агоніст-стимульовану секрецію ацинарних клітин підшлункової задози, що є енергозатратним процесом та потребує достатньої кількості субстратів окиснення. За окиснення піруват холецистокінін значно підвищує швидкість дихання ацинарних клітин підшлункової залози. Проте недостаньо зрозуміло, як впливає інсулін на роботу мітохондрій у спокої та за активації секреції. Експерименти проводили на щурях самцях лінії Вістар масою 250–300 г, що перебували на стандартрій дієті та голодували 12 годин перед експериментом. Панкреатичні ацинуси ізолювали з колагеназою. Базальне і FCCP-стимульоване дихання ізольованих панкреатичних ацинусів щурів вимірювали за допомогою електрода Кларка. Статистичну вірогідність (P) різниці між середніми арифметичними оцінювали парним t-тестом або двофакторним дисперсійним аналізом з повторами та post-hoc тестом Turkey. Адаптаційну здатність мітохондрій оцінювали за максимальною швидкістю роз'єднаного дихання. Адаптаційна була вищою, коли як субстрат окиснення використовували піруват (2 ммоль/л), у порівнянні із глюкозою (10 ммоль/л). Інкубація з інсуліном (100 нмоль/л) впродовж 20 хв підвищувала швидкість базального дихання та адаптаційну здатність мітохондрій панкреатичних ацинусів за використання глюкози, але не піруват. Холецистокінін (0.1 нмоль/л, 30 хв) збільшував швидкість базального та максимального роз'єднаного дихання ацинарних клітин за окиснення піруват, але інсулін повністю нівелював це зростання адаптаційної здатності мітохондрій. Отже, інсулін підвищує окиснення глюкози у ацинарних клітинах підшлункової залози у стані спокою, але пригнічує окиснення піруват за стимуляції секреції холецистокініном. Для з'ясування механізмів впливу інсуліну на метаболізм піруват у цих клітинах необхідні подальші дослідження.

Ключові слова: панкреатичні ацинуси, інсулін, холецистокінін, глюкоза, піруват