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EFFECTS OF ACUTE ALCOHOL INTOXICATION ON THE RESPIRATION AND DEHYDROGENASE ACTIVITY OF RAT PANCREATIC ACINI

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The exocrine function of pancreatic acini cells is a highly energy-intensive process. A cell always needs to maintain a stable level of ATP balancing between states of activation and rest. Low-energy is one of the possible mechanisms contributing to the development of pancreatic diseases. The most commonly encountered disease of the pancreas is acute pancreatitis. It is known that excessive alcohol consumption causes the development of pancreatitis. The pathogenesis of this disease is linked to the cellular loss of energy, but the mechanism of alcohol's effect on the mitochondria in pancreatic acini is unclear. This study's main aim is to assess the impact of acute alcohol administration on the mitochondrial function of rat pancreatic acini.

Wistar rats were administered ethanol (6 g/kg body weight) by oral gavage for 3 h before the experiment. A suspension of isolated pancreatic acini was obtained following collagenase digestion. Respiration of isolated pancreatic acini was studied with a Clark electrode. The maximal respiration rate was studied at different concentrations of protonophore FCCP (0.5–2 μM) in solutions containing glucose combined with oxidative substrates (pyruvate and glutamine, monomethyl-succinate or dimethyl-α-ketoglutarate). Dehydrogenase activity was measured by colorimetric method.

Ethanol administration caused a significant increase in the activity of pyruvate dehydrogenase. It was confirmed that FCCP induced an increase in the respiration rate of pancreatic acinar cells in each experimental group. The addition of 1.5 μM FCCP reduced the respiration rate of pancreatic acini during the oxidation of glucose and monomethyl succinate or dimethyl-α-ketoglutarate, but not during the oxidation of glucose, pyruvate and glutamine substrates. The administration of ethanol had no impact on the basal or FCCP-uncoupled respiration of isolated pancreatic acini. The observed data are consistent with the findings of other researchers. However, alcohol exposure is not sufficient to cause mitochondrial damage in pancreatic acinar cells.

In conclusion, acute ethanol administration does not cause mitochondrial dysfunction in the pancreas of rats but causes an increase in pyruvate dehydrogenase activity.

Keywords: pancreatic acini, alcohol, dehydrogenase, glucose, pyruvate

Introduction

Ethanol consumption is a major factor causing acute pancreatitis. The pathogenesis of pancreatitis is considered to be associated with mitochondrial damage [1]. At the moment it is not clearly understood if mitochondrial damage is the cause or effect of pancreatic acinar cell damage in the early pancreatitis events. It has been established that ethanol *in vitro* leads to changes in the functioning of mitochondria [20], Ca^{2+} signalling [6, 19] and the generation of ROS [8] in pancreatic acini. Nevertheless, these outcomes were not verified *in vivo*, and it is most likely that ethanol requires additional factors to cause pancreatitis [12, 15, 17]. In our previous study, we found that while a combination of ethanol and cholecystokinin administration to rats caused a significant decrease of both basal and uncoupled respiration rate of isolated pancreatic acini,

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ethanol alone did not affect the uncoupled respiration and its effect on basal oxygen consumption was less convincing [16] due to a complex experimental design.

The aim of this study was thus to re-assess the effect of acute ethanol administration on mitochondrial respiration of rat pancreatic acini, supported by the dehydrogenase activity investigation.

Materials and Methods

All manipulations with animals have been performed by the EU Directive 2010/63/EU for animal experiments and laws of Ukraine. Experimental protocols were approved by the Animal Care and Use Committee of Ivan Franko National University of Lviv.

Experiments were carried out on Wistar white male rats (250–300 g). All animals were kept under the standard conditions of a vivarium at room temperature (18–20 °C) on a standard diet. Three hours before decapitation the animals were administered ~ 4 ml of water (control) or 40 % alcohol solution (6 g per kg of animal weight).

A suspension of isolated pancreatic acini was obtained following collagenase digestion (Sigma, type IV, 0.2 mg/ml) according to the previously reported method [14].

Acini were obtained and stored in extracellular solution containing (mM): 140.0 NaCl, 4.7 KCl, 1.3 $CaCl_2$, 1.0 $MgCl_2$, 10.0 HEPES, 10.0 glucose, 2.0 glutamine, 2.0 sodium pyruvate, 0.01 % (wt/vol) soybean trypsin inhibitor, 0.25 % (wt/vol) BSA and MEM amino acid supplement; pH 7.4 (NaOH). Cell viability was evaluated with the trypan blue test (0.1 %). Cell counting was performed using a haemocytometer.

Oxygen consumption was measured with a Clark oxygen electrode at 37 °C using SI929 6-channel Oxygen Meter (Strathkelvin). Before respiration measurement, suspension of pancreatic acini was pre-incubated (15 min, 37 °C) in respective solutions with glucose (10 mM) with oxidative substrates (2 mM pyruvate and 2 mM glutamine or 2 mM monomethyl-succinate or dimethyl-α-ketoglutarate). Maximal uncoupled respiration was studied with protonophore FCCP added step-wise to reach the final concentrations 0.5, 1, 1.5, and 2 μM as previously described [15].

The activity of selected dehydrogenases was studied by a colorimetric method with nitroblue tetrazolium [11, 22]. Cells or lysate were incubated with 200 μl of either the complete assay mixture or the assay mixture without substrate for 40 min at 37 °C [11, 22]. A separate aliquot of acini in suspension was homogenized in a lysis solution, containing 0.1 % Triton X-100 in addition to the basic solution. Enzyme activity was studied with colorimetric assay using a DENOVIX spectrophotometer DS-11 FX+ in the 1 cm cuvette at a wavelength of 540 nm at 37 °C. Activity is calculated by subtraction of the absorbance of the containing the blank control (without lysate).

All reagents 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 acini from different animals ($n \geq 6$). Statistical analysis was performed using Microsoft Excel software. Data are presented as mean ± standard error of mean. Statistical significance (P) of the difference between the means was assessed in each case with a paired t-test.

Results and Discussion

In the first experiment, the suspension of isolated pancreatic acini was incubated for 15 min at 37 °C in the basic solution containing glucose and some of the substrates: monomethyl succinate, dimethyl-α-ketoglutarate, pyruvate and glutamine.

Upon glucose and monomethyl succinate oxidation, adding 0.5 and 1 μM FCCP to the respiration chamber caused an increase in the respiration rate to 1.8±0.24 and 2.4±0.27 r.u., re-

spectively. At higher concentrations, FCCP caused a gradual decrease in respiration rate during the oxidation of glucose and monomethyl succinate or dimethyl-α-ketoglutarate (Fig.1 *A* and *B* P<0.001) which is consistent with previous data [15]. When glucose, pyruvate and glutamine were used as substrates, all concentrations of FCCP increased the respiration rate of isolated pancreatic acini (Fig.1 *C*, P<0.05 – 0.001). There was no statistically significant difference between the alcohol and control groups. Alcohol caused no effect on the basal and maximal FCCP-uncoupled respiration of isolated pancreatic acini when tested substrates were used (Fig. 2). These results suggest that acute alcohol administration does not damage mitochondria in pancreatic acinar cells, which might lead to acute pancreatitis [18]. Only a combination of ethanol with an additional factors impairs pancreatic acinar cells in animal models [9, 12, 16].

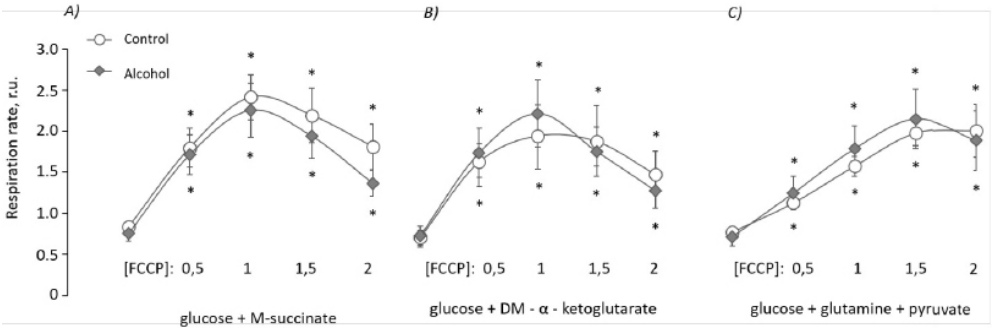

Fig. 1. The effects of *in vivo* ethanol administration (3 h) to rats on respiration of pancreatic acini: [glucose] = 10 mM, [pyruvate] = 2 mM, [glutamine] = 2 mM, [monomethyl-succinate] = 2 mM, [dymetyl-α-ketoglutarate] = 2 mM, [FCCP] = 0.5–2 μM; * – statistically significant difference compared to basal rate with P<0.05; Mean ± SEM, n=6–8

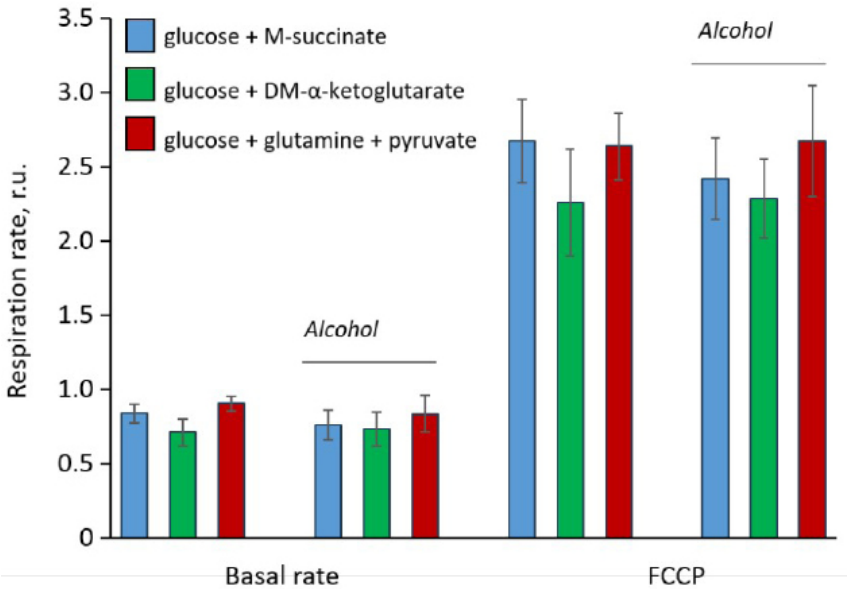

Fig. 2. The effects of *in vivo* ethanol administration (3 h) to rats on basal and maximal FCCP-uncoupled respiration of isolation pancreatic acini: [glucose] = 10 mM, [pyruvate] = 2 mM, [glutamine] = 2 mM, [monomethyl-succinate] = 2 mM, [dymethyl-α-ketoglutarate] = 2 mM, [FCCP] = 0.5–2 μM; Mean ± SEM, n=6–8

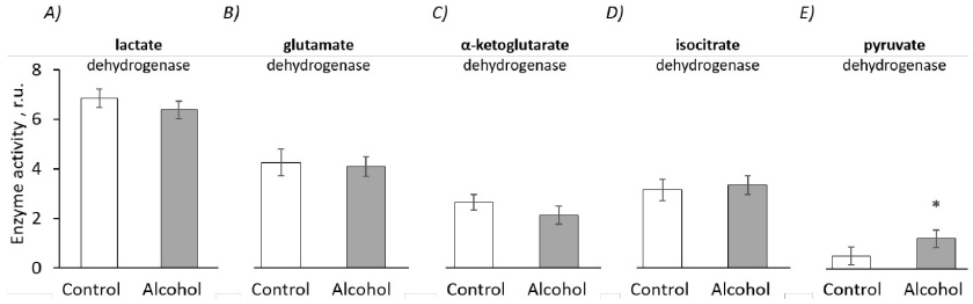

Fig. 3. The effects of *in vivo* ethanol administration (3 h) to rats on enzyme activity of pancreatic acini: *A* – lactate dehydrogenases, *B* – glutamate dehydrogenases, *C* – α-ketoglutarate dehydrogenases, *D* – isocitrate dehydrogenases, *E* – pyruvate dehydrogenases; * – statistically significant difference compared to control with P<0.05; Mean ± SEM, n=7–8

It is known that ATP levels decrease in pancreatic acinar cells following various pancreatic injuries [5], but the mechanism has not been fully studied. In the next experiment, we investigated the activity of dehydrogenases, which play an essential role in producing ATP in cells.

It was found that alcohol administration caused a significant 2.3-fold increase in the pyruvate dehydrogenase activity in isolated pancreatic acini (1.22±0.27 *vs* 0.52 ± 0.07 r.u., Fig. 3, *E,* P=0.02). The activities of other tested dehydrogenases were unchanged.

It is known that pyruvate oxidation under the influence of secretagogues occurs through the activation of pyruvate dehydrogenase by Ca^{2+}. *In vitro* studies have shown that only high concentrations of alcohol lead to short-term alterations in Ca^{2+} levels in pancreatic acini [4]. Other studies have shown that ethanol concentrations ranging from 1 to 50 mM trigger the release of Ca^{2+} from stores in pancreatic acini [7]. In light of these, it seems that alcohol alone in a studied concentration should not be enough to activate pyruvate dehydrogenase. However, non-oxidative ethanol metabolism, which involves the formation of ethyl esters of fatty acids (FAEE), is dominant in pancreatic acini *in vivo* [9]. In humans, 2 mM FAEE was detected at a concentration of 30 mM alcohol in the blood [2]. Data from *in vitro* experiments show that 10–100 μM FAEE induces a rise in intracellular [Ca^{2+}] [4, 13]. Our previous experiments have shown that pyruvate supplementation improves the survival of pancreatic acini when they are exposed to ethanol and CCK [16]. We can assume that alcohol consumption to leads the formation of FAEE, which triggers Ca^{2+} release and the activation of the pyruvate dehydrogenase in rats. Consequently, acute alcohol administration does not impact the maximal respiration rate of pancreatic acinar cells upon the oxidation of each experimental substrate *in vivo*. Only the activity of pyruvate dehydrogenase increases in pancreatic acini under the influence of alcohol, unlike other analyzed dehydrogenases.

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ВПЛИВ ГОСТРОЇ АЛКОГОЛЬНОЇ ІНТОКСИКАЦІЇ НА ДИХАННЯ ТА ДЕГІДРОГЕНАЗНУ АКТИВНІСТЬ ПАНКРЕАТИЧНИХ АЦИНУСІВ ЩУРІВ

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Екзокринна функція клітин ацинусів підшлункової залози є енергозалежним процесом. Клітині постійно необхідно підтримувати стабільний рівень АТФ, балансуючи між станами активації та спокою. Низький рівень енергетичного забезпечення є одним із потенційних механізмів, які сприяють розвиткові захворювань підшлункової залози. Найпоширенішим захворюванням підшлункової залози є гострий панкреатит. Відомо, що надмірне споживання алкоголю є чинником розвитку панкреатиту. Патогенез цього захворювання пов'язують із порушенням клітинної енергетики, проте механізм дії етанолу на мітохондрії ацинарних клітин підшлункової залози залишається недостатньо вивченим. Це дослідження мало на меті оцінити вплив одноразового введення алкоголю на функціонування мітохондрій ацинарних клітин підшлункової залози щурів.

Щурам лінії Wistar перорально вводили алкоголь (6 г/кг маси тварини) одноразово за 3 год до проведення експерименту. Панкреатичні ацинуси ізолювали за допомогою колагенази. Дихання ізольованих панкреатичних ацинусів досліджували з використанням електроду Кларка. Максимальну швидкість дихання оцінювали, використовуючи різні концентрації протонофору FCCP (0,5–2 мкМ) у середовищі, що містило глюкозу в комбінації з іншими окислювальними субстратами (піруватом і глутаміном, монометилсукцинатом або диметил-α-кетоглутаратом). Активність дегідрогенази вимірювали колориметричним методом.

Введення алкоголю (3 год) щурам спричинило статистично достовірне зростання активності піруватдегідрогенази. Було підтверджено, що FCCP призвів до збільшення швидкості дихання ацинарних клітин підшлункової залози в кожній експериментальній групі. Додавання 1,5 мкМ FCCP знизило швидкість дихання панкреатичних ацинусів під час окиснення глюкози та монометилсукцинату або диметил-α-кетоглутарату, але не під час окиснення субстратів глюкози, пірувату і глутаміну. Введення етанолу не вплинуло на базальну та максимальну швидкість дихання ізольованих ацинусів підшлункової залози. Отримані дані узгоджуються з результатами інших досліджень. Очевидно, що впливу тільки лиш алкоголю недостатньо, щоби спричинити мітохондріальне пошкодження ацинарних клітин підшлункової залози.

Отже, одноразове введення алкоголю щурам не спричинило мітохондріальної дисфункції в ацинарних клітинах підшлункової залози щурів, проте достовірно підвищувало активність піруватдегідрогенази.

Ключові слова: панкреатичні ацинуси, алкоголь, дегідрогенази, глюкоза, піруват