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Small molecules released from islets of Langerhans determined by liquid chromatography – mass spectrometry V.2

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Human Islet Research N...

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Abstract

Islets of Langerhans are the endocrine tissue within the pancreas that secrete hormones for maintenance of blood glucose homeostasis. A variety of small molecules including classical neurotransmitters are also released from islets. While the roles of most of these small molecules are unknown, some have been hypothesized to play a critical role in islet physiology. To better understand their role on islet function, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed to separate and quantify 39 small molecules released from islets. Benzoyl chloride derivatization of analyte molecules was used to impart retention and facilitate electrospray ionization efficiency. Separation was achieved on a 2.1 × 150 mm column packed with 2.7 µm core–shell C_{18}particles. Calibration curves showed excellent linearity between the concentration and analyte response, with relative standard deviations of the analyte responses below 15% and limits of detection from 0.01–40 nM. The method was applied to examine small molecules released from murine and human islets of Langerhans after static incubation and perfusion with glucose. Results showed a decrease in secretion rates with increasing glucose concentration for most of the analytes. Secretion rates were found to be higher in human islets compared to their murine counterpart. This method will be useful in understanding the roles of small molecules in biological systems.

Guidelines

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Anal. Methods, 2021, 13 , 3614 —3619.

https://pubs.rsc.org/en/content/articlelanding/2021/AY/D1AY00703C LINK

Materials

Chemicals and reagents:

Sodium hydroxide (NaOH)(Catalog# 72068)was purchased from EMD Millipore (San Diego, CA).

Dextrose(Catalog# D16-1) and **tricine**(Catalog# AC172640250) were from Fisher Scientific (Pittsburgh, PA).

LC grade acetonitrile (ACN)(Catalog# 97065-022) was from VWR (Radnor, PA).

d4-Acetylcholine (d4-ACh)(Catalog# D-2558) was from CDN Isotopes Inc. (Pointe-Claire, QC).

All solutions were made with **HPLC grade submicron filtered water**(Catalog# W5SK-1) (Fisher Chemical, Fair Lawn, NJ).

Stock solutions of serine (Ser), threonine (Thr), asparagine (Asn), glutamine (Gln), alanine (Ala), histidine (His), aspartate (Asp), tyrosine (Tyr), γ-aminobutyric acid (GABA), valine (Val), methionine (Met), leucine (Leu), phenylalanine (Phe), tryptophan (Trp), arginine (Arg), taurine (Tau), glycine (Gly), glutamate (Glu), dopamine (DA), serotonin (5-HT), proline (Pro), *trans*-4-hydroxy proline (Hyp), cysteine (Cys), lysine (Lys), isoleucine (Ile), α-aminobutyric acid (α-ABA), β-aminobutyric acid (β-ABA), β-homoserine (β-HSer), tyramine (TryA), citrulline (Cit), kynurenine (Kyn), 2-aminoadipic acid (Aad), ornithine (Orn), histamine (Hist), 5-hydroxytryptophan (5-HTP), *N*-acetylcysteine (NAC), epinephrine (Epi), and acetylcholine (ACh) were made in LC water and diluted to working concentrations using a balanced salt solution (BSS) containing 25 mM tricine, 125 mM NaCl, 5.9 mM KCl, 1.2 mM $MgCl_2$, 2.4 mM $CaCl_2$, and various glucose concentrations as described in the text, and the pH was adjusted to pH 7.4 with NaOH.

Troubleshooting

Safety warnings

Safety information

Acetonitrile (ACN)

Hazard statements:
Highly flammable liquid and vapor.
Harmful if swallowed, in contact with skin or if inhaled.
Causes serious eye irritation.

Precautionary statements:
Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
Wear protective gloves/protective clothing/eye protection/face protection.
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
IF exposed or concerned: Immediately call a POISON CENTER/doctor

Safety Data Sheet

Methods

1 Benzoyl chloride derivatization

For production of calibration curves, 100 µL of a standard mixture of small molecules (concentrations given in **Section 3.3 in the original publication)** in BSS was mixed with 50 µL of 100 mM sodium carbonate (pH 9.2) and 50 µL 2% BzCl (by volume in ACN). The mixture was vortexed and allowed to react for 30 s before quenching with 50 µL of IS (composition described below). The ratio of sample : carbonate buffer : BzCl : IS was always 2 : 1 : 1 : 1 (v/v/v/v). IS were produced by derivatization of the small molecules with ^{13}C benzoyl chloride using the method described above. Because ACh did not derivatize, d4-ACh was spiked into the IS solution to a final concentration of 100 nM. To obtain desalted components for building the MRM method described in **LC-MS instrumentation Section,** analytes and IS were extracted with dichloromethane (DCM), followed by evaporation with N_2 gas and reconstitution in 20% ACN containing 0.1% formic acid (FA).

2 LC-MS instrumentation

LC-MS experiments were performed using a **Thermo Scientific (Waltham, MA) Vanquish Flex UHPLC system** with a Split Sampler Module and a **Thermo Scientific TSQ Quantis triple quadrupole mass spectrometer**. 10 mM ammonium formate with 0.1% FA was used as mobile phase A (MPA) and ACN containing 0.1% FA used as mobile phase B (MPB). Separations were performed on a 2.1 × 150 mm, 2.7 µm, **160 Å pore ES-C18 column** (Halo Peptide, Mac-Mod Analytical, Chadds Ford, PA) used with a 2.1 × 5 mm, 2.7 µm, **160 Å pore ES-C18 guard column** (Mac-Mod Analytical). The injection volume was 5 µL and the mobile phase flow rate was 0.25 mL min^{-1}. The column temperature was held constant at 25 °C in still air mode while the autosampler was set to 4 °C .

The TSQ Quantis mass spectrometer was operated in MRM mode. Electrospray settings were: spray voltage of 3500 V, sheath gas at 4.19 L min^{-1}, auxiliary gas of 6.4 L min^{-1}, sweep gas 1.5 L min^{-1}, ion transfer tube temperature of 300 °C , and a capillary temperature of 275 °C . For MS/MS optimization, desalted sample was directly infused into the mass spectrometer at a flow rate of 50 µL min^{-1}. The most intense and consistent fragments were used for building the MS/MS method for each analyte and IS. During LC-MS/MS runs, a time managed MRM was performed where the MRM transition for specific analytes were examined in a retention time (RT) window, defined as the RT ± 0.50 min (**Table S-1**). A dwell time of 50 ms was used for each transition.

3 **Isolation and culture of islets of Langerhans**

All animal experiments were performed under guidelines approved by the Florida State University Animal Care and Use Committee, protocol 202000078. Murine islets were obtained by digesting the pancreas from two male CD-1 mice (20–40 g) with collagenase as previously described[1,2]. The islets from both mice were combined and incubated at 37 °C with 5% CO_2 in **RPMI 1640**(Cat# 10-040-CV, Corning, Manassas, VA) containing 11 mM glucose, l-glutamine, 10% fetal bovine serum, 100 U mL^{-1}penicillin, 100 µg mL^{-1}streptomycin, and 10 µg mL^{-1}gentamycin. Islets were used the day after isolation.

Human islets were purchased from Prodo Laboratories (Aliso Viejo, CA) from donors with no history of diabetes. The islets were recovered according to the protocol established by Prodo Laboratories, followed by incubation in Prodo Islet Media Standard PIM(S) at 37 °C and 5% CO_2 for at least 1 day. Human islet samples were from deidentified cadaveric organ donors and therefore exempt from Institutional Review Board approval. Islets from two donors were used in separate experiments and donor characteristics are shown in **Table S-2**. Islets from Donor 1 were used in a static incubation experiment while islets from Donor 2 were used in a perfusion experiment.

4 **Static incubation of islets**

For static incubation experiments, 20 human or murine islets were removed from culture media and rinsed three times with prewarmed BSS containing 1 or 3 mM glucose, respectively. The rinsed islets were then placed in a 250 µL microcentrifuge tube containing 100 µL of the same BSS solution and incubated at 37 °C with 5% CO_2. After one hour, 90 µL of the supernatant was removed and replaced with 90 µL BSS containing 22.2 mM glucose for human islets and 22 mM glucose for murine islets to bring the final glucose concentration to 20 mM. The islets were then incubated for 1 h at 37 °C with 5% CO_2, after which another 90 µL of the supernatant was removed. Supernatant from both incubations were derivatized separately as described in **Benzoyl chloride derivatization Section.**

5 **Dynamic perfusion of islets**

The microfluidic device fabrication, setup, and characterization are similar to that described elsewhere[3] and given in more detail in the ESI.† Prior to each use of the microfluidic device, the system was conditioned by flowing RPMI media with serum for

30 min and stopping the flow for 30 min. This procedure was followed by 30 min of perfusion with BSS containing 1 or 3 mM glucose for human or murine islets, respectively. 25 islets were held in a dish containing 1 or 3 mM prewarmed glucose in BSS for 10 min in the incubator. The islets were then loaded into the islet chamber and allowed to settle to the bottom. The islet chamber was sealed with PCR film, placed in the incubator for 10 min, after which the input and output tubing were connected. BSS with 1 or 3 mM glucose was delivered to the islets at 5 µL $min^{-1}$for 30 min prior to fraction collection. Islets were perfused with low glucose (1 or 3 mM) for 10 min, followed by high glucose (20 mM) for 30 min, and low glucose (1 or 3 mM) for another 10 min. The fractions were collected in a 96-well plate, derivatized, and injected into the LC-MS.

6 **Data analysis**

Chromatograms were analyzed using **Xcalibur (Thermo Scientific) software**. The software was used to integrate the peak area that coincided with the retention time window for each analyte and IS. Calibration curves were constructed by plotting the ratio of the average blank-subtracted analyte : IS peak area for 3 injections against the concentration of analyte injected. Error bars in all plots are equal to ±1 standard deviation (SD) unless otherwise noted. Linear least-squares were used to fit the data and the resulting regression equations were used to calculate the unknown concentrations of analytes from islet samples. LOD for each analyte were calculated using 3 times the SD of the blank peak area divided by the slope of each calibration curve. Comparison of sample means was performed using a paired two-tailed *t*-test, unless otherwise noted, with significance determined when $p < 0.05$. Resolution (R_S) between peaks was calculated using the difference in retention times and the average of the peak widths at baseline.