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Micellar electrokinetic chromatography method for measuring amino acid secretions from islets of Langerhans

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Abstract

Islets of Langerhans are responsible for maintaining glucose homeostasis through regulated secretion of hormones and other factors. It is hypothesized that amino acids secreted from islets play a critical role in cell functionality and viability. For example, glutamate and gamma-aminobutyric acid (GABA) have been proposed to work as paracrine signaling molecules within islets to coordinate the release of hormone secretion; other amino acids, such as glutamine, leucine, alanine, and arginine have been shown to stimulate or potentiate glucose-stimulated insulin secretion. To characterize the potential roles that these small molecules may play in islet physiology, derivatization of amino acids in high salt buffers commonly used in islet experiments with naphthalene-2,3-dicarboxaldehyde and MEKC separation conditions were optimized. The optimized conditions used D-Norvaline as the internal standard and allowed quantification of 14 amino acids with LODs ranging from 0.2 – 7 nM. The RSD of the migration times were 0.04 – 0.54% and the RSD of the peak areas were 0.2 – 5.8% for the various amino acids. The effects of glucose and 2,4-dinitrophenol on amino acid secretions from islets were tested and a suppressive effect of glucose on GABA release was observed, likely acting through ATP inactivation of glutamate decarboxylase.

Keywords

capillary electrophoresis; laser-induced fluorescence (LIF); naphthalene-2,3-dicarboxaldehyde (NDA); glucose; 2,4-dinitrophenol

1 Introduction

Islets of Langerhans constitute the endocrine portion of the pancreas and are responsible for helping to maintain glucose homeostasis through regulated secretion of numerous factors, most notably, peptide hormones [1]. In addition to hormones, islets also release many small molecules [2-4]. Whereas the role of peptide hormones is well established, the role of small molecule secretion from islets is not, but they have been hypothesized to play a critical role in cell functionality [2-4].

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Islet α - and β -cells compose more than 90% of the islet and are responsible for the release of glucagon and insulin, respectively. These peptides help regulate glucose homeostasis and are also known to act on neighboring cells in a paracrine manner [5]. Similar paracrine effects have been proposed for some of the small molecules released from islets, such as glutamate (Glu) and gamma-aminobutyric acid (GABA) [3,4], which are major excitatory and inhibitory neurotransmitters, respectively, in the central nervous system [6,7]. Glutaminase, the enzyme responsible for converting glutamine (Gln) to Glu, has been detected in α -cells of the pancreas, as well as the intrapancreatic ganglia that innervate the islets [8]. Combined with the detection of ionotropic Glu receptor subtypes in islets, it has been hypothesized that Glu secreted from α cells or the ganglia may modify insulin secretion [8]. Glutamate decarboxylase (GAD) and GABA have been found in β - cells [9]; GABA has been shown to inhibit glucagon secretion from α -cells by acting on GABA_A receptors [10]. Therefore, these two small molecules have been proposed to work as paracrine signaling molecules within the islet to help coordinate the release of hormone secretion in islets [3,4,8,11]. Besides these two, other amino acids, such as Gln, leucine (Leu), alanine (Ala), and arginine (Arg) have been shown to stimulate or potentiate glucose-stimulated insulin secretion [12-15]. To characterize the potential roles that these small molecules may play in islet physiology, methods to measure their release with high sensitivity are required.

There have been several approaches taken for detection of amino acids [16-18]. The most common approaches used for separation of amino acids are HPLC and CE. Compared to HPLC, CE offers advantages such as faster analysis speeds and higher separation efficiencies. Coupled with laser-induced fluorescence (LIF) detection, low mass detection limits are also achieved, which enable measurements of time-resolved samples [19,20]. Because most amino acids are not natively fluorescent, detection by LIF requires covalent modification of the analytes with a fluorescent tag. Currently, there are several commercially-available labeling reagents such as naphthalene-2,3-dicarboxaldehyde (NDA) [21,22], o- phthalaldehyde [23,24], and 4-fluoro-7-nitro-2,1,3-benzoxadiazole [25]. NDA reacts quickly with primary amines in the presence of cyanide (or another nucleophile) to produce stable (cyano-benz(f)-isoindole) derivatives that have fluorescence quantum yields ranging from 0.5 – 0.8 [21]. This labeling scheme has been successfully used to measure amino acids by CE-LIF with LODs reported from 2-100 nM [19,20].

In this work, we developed an optimized derivatization and separation protocol to measure amino acids that were released from islets of Langerhans into high salt buffers that are compatible with cell culture. An internal standard, D- norvaline (D-Nva), was added to the amino acid mixtures and used to normalize injection volume variations. This method allowed the quantification of 14 amino acids with LOD ranging from 0.2 – 7 nM. The RSD of the migration times were 0.04 - 0.54% and the RSD of peak areas were 0.2 - 5.8%, indicating the robustness and stability of this protocol. The effects of glucose on amino acid secretions from islets were tested, and the suppressive effect of glucose on GABA release was observed, similar to what has been observed elsewhere [26-28]. The method described here also has the potential for characterizing secretions from other tissue samples for understanding the role amino acids may play as signaling molecules.

2 Materials and methods

2.1 Chemicals and reagents

Dextrose, sodium phosphate monobasic, sodium tetraborate, and HEPES were from Fisher Scientific (Pittsburgh, PA). Cosmic Calf Serum was from HyClone Laboratories (South Logan, Utah). Sodium hydroxide (NaOH) was purchased from EMD Chemicals (San Diego, CA). ACN was from Avantor Performance Materials (Center Valley, PA). Collagenase P was obtained from Roche Diagnostics (Indianapolis, IN). RPMI 1640 was from Mediatech (Manassas, VA). Gentamicin was from Lonza (Walkersville, MD). All other chemicals were from Sigma-Aldrich (St. Louis, MO) unless otherwise noted. All solutions were made with ultrapure DI water (NANOpure® Diamond system, Barnstead International, Dubuque, IA).

2.2 Amino acid derivatization

Stock solutions of serine (Ser), threonine (Thr), asparagine (Asn), Gln, Gly, Ala, histidine (His), Glu, aspartate (Asp), tyrosine (Tyr), GABA, valine (Val), methionine (Met), isoleucine (Ile), Leu, phenylalanine (Phe), tryptophan (Trp), and Arg were made in DI water and diluted to working concentrations using a balanced salt solution (BSS) containing 125 mM NaCl, 5.9 mM KCl, 1.2 mM MgCl₂, 2.4 mM CaCl₂, and 25 mM HEPES at pH 7.4. The concentrations of the amino acids in the calibration solutions ranged from 5 - 2000 nM in BSS. A 5 mM NDA solution was prepared in a 50:50 (v:v) mixture of ACN and 15 mM borate buffer at pH 9.0, and a 20 mM cyanide solution was prepared in 15 mM borate buffer at pH 9.0. For each derivatization reaction, 90 µL of BSS containing the standards or islet secretions were mixed with 10 µL of 10 µM internal standard, D-Nva, in BSS, 10 µL of 5 mM NDA, and 10 µL of 20 mM cyanide. The mixture of the 18 amino acids and the internal standard was then kept in the sample tray of the CE instrument where the derivatization temperature was controlled. Derivatization conditions were optimized by testing different reaction times and temperatures, ranging from 5 - 120 min and 25 - 55 °C, respectively.

2.3 CE-LIF instrument operation

CE experiments were performed on a 25 or 50 µm id, 30 or 60 cm (20 or 50 cm effective) length of fused-silica capillary (Polymicro Technologies, Phoenix, AZ) using a Beckman P/ACE MDQ system (Beckman-Coulter, Fullerton, CA) equipped with an LIF module. A 100 mW, 450 nm laser diode (Aixiz, Houston, TX) was used as the excitation source with a 480 ± 20 nm bandpass filter prior to detection. Data were acquired at 4 Hz.

At the start of each day, the capillary was rinsed with 1 M NaOH, 0.1 M NaOH, and deionized water for 15 min at 50 psi. The capillary was then conditioned with run buffer for 15 min at 50 psi. Between analyses, the capillary was rinsed with 0.1 M NaOH followed by the run buffer, each for 10 min at 50 psi. After derivatization, samples were pressure injected at 1 psi for 15 s, followed by a wait step in DI water for 6 s to remove excess sample from the outside of the capillary. The capillary was then moved to the separation buffer vials to commence electrophoresis using the optimized separation conditions described in Section 3.2.

2.4 Isolation and incubation of islets of Langerhans

All experiments were performed under guidelines approved by the Florida State University Animal Care and Use Committee, protocol #1235. Islets were obtained by digesting the pancreas from 20 – 40 g male CD-1 mice with collagenase as previously described [29]. Isolated islets were cultured in RPMI 1640 media (Mediatech, Inc., Manassas, VA) containing 11 mM glucose, 10% serum, 100 units mL⁻¹ penicillin, 100 µg mL⁻¹ streptomycin, and 10 µg mL⁻¹ gentamicin at 37 °C in the presence of 5% CO₂. Islets were used within 5 days after isolation.

Prior to experiments, 25 islets were picked from the culture media, rinsed in the incubation buffer (BSS with either 3 or 20 mM glucose) 4 times (2 mL each), and then incubated in 120 µL of BSS containing either 3 or 20 mM glucose, with or without 100 µM 2,4-dinitrophenol (DNP). After 1 h of incubation, 90 µL of the BSS containing the secretions was collected and derivatized. A control experiment was performed by derivatization of 90 µL BSS with either 3 or 20 mM glucose.

2.5 Data analysis

Electropherograms were analyzed using 32KaratTM software. For quantification, peak areas were background subtracted and normalized by the background subtracted peak area of D-Nva. Calibration curves were obtained by plotting the normalized peak areas of each amino acid against its concentration. Regression equations were calculated by the least-squares linear regression method. Throughout the manuscript, the concentrations of amino acids given are those of the amino acids in the BSS prior to the addition of derivatization reagents. LOD was determined as the concentration of amino acid that produced a signal three times the standard deviation of the peak area from a background sample injection, which contained only derivatized D-Nva in BSS. Each secretion sample was injected three times and results are presented as mean ± S.E.M. for 3 replicate runs. A two-tailed Student's t-test was used to compare different derivatization or incubation conditions. A one-tailed Student's t-test was used to compare the effects of glucose or DNP on amino acid secretion. A p-value < 0.05 was considered to be statistically significant. Percent recoveries of Glu, Gln, and GABA were calculated by spiking in 1 µM standards into islet secretions.

3 Results and discussion

3.1 Optimization of derivatization conditions

The measurement of amino acid neurotransmitters by CE-LIF has been performed for decades with many different conditions utilized and applied to either method development, *in vitro*, or *in vivo* applications [30-34]. We thought to modify these well-established procedures to make them amenable to measurement of amino acid release from islets of Langerhans.

A mixture of 18 amino acids plus the internal standard (1 µM each before derivatization) was used to optimize derivatization conditions with NDA. Before the separation conditions were optimized, only Ser, Glu, Gln, Val, Ile, Leu, GABA, and D-Nva were baseline resolved; therefore, the peak areas of these amino acids were chosen to optimize the

derivatization conditions. To minimize dilution, a small relative volume of the NDA, cyanide, and internal standard was required and a volumetric ratio of 9:1:1:1 (sample:NDA:cyanide:D-Nva) was chosen. However, this produced a solution that was ~83% BSS, which is a high ionic strength solution. A high conductivity sample can lead to band broadening due to anti-stacking [35], but with optimized injection conditions of 15 s at 1 psi (1.86 nL plug volume on a 50 cm effective length capillary), no substantial broadening was observed.

To optimize derivatization time of the amino acids with NDA, the 9:1:1:1 mixture was stored in the sample tray of the CE instrument and the reaction was allowed to proceed for 5, 10, 20, 60, and 120 min at 25 °C (n = 3 for each time point). The resulting solutions were then injected and separated with the average peak area in the resulting electropherograms shown as a function of reaction time in Figure 1A. At a reaction temperature of 25 °C, the average peak areas of most amino acids increased during the first 20 min. After 20 min, the areas reached a plateau, indicating the reaction was complete. As a result, 20 min was chosen as the optimum reaction time. To test the effects of temperature on the derivatization reaction, the reaction time was fixed at 20 min while reaction temperatures from 25 - 55 °C were tested. As can be seen in Figure 1B, the average peak areas were similar at different temperatures tested (n = 3 for each temperature), indicating no effect of temperature at these conditions. The optimized derivatization temperature was set at 25 °C to minimize evaporation.

3.2 Optimization of separation conditions

After derivatization, the amino acids have similar electrophoretic mobilities making separation by conventional CE difficult. To overcome this problem, MEKC is often used. SDS is a common buffer additive in MEKC separations for NDA-labeled amino acids, and its concentration has also been optimized [19, 34]. However, because of the high concentration of salt in the sample, optimization of buffer composition, concentration, and pH, as well as capillary dimensions, was performed to resolve the largest number of amino acids as possible.

Using a series of derivatized amino acids, the effects of capillary length and id on the resolution were tested using a separation buffer of 15 mM phosphate at pH 8 with 30 mM SDS. Using a mixture of 10 amino acids with the internal standard, separation lengths of 20 and 50 cm were tested. The shorter separation length allowed a faster separation, but failed to achieve baseline resolution of many of the compounds. After determining the 50 cm length was ideal, an additional 8 amino acids were added to the mixture, and the optimum capillary id was tested. The capillary conditions that allowed the largest number of amino acids to be baseline resolved were found to be 50 cm separation length with a 25 µm id. Once the capillary dimensions were optimized, the effect of buffer composition on the separation and resolution of a mixture of 19 amino acids was examined. The buffers tested were 15 mM phosphate (pH = 8) with 30 mM SDS; 15 mM phosphate (pH = 8) with 30 mM SDS and 5 mM hydroxypropyl-β-cyclodextrin (HP-β-CD); 75 mM borate (pH = 9) with 70 mM SDS; 20 mM borate (pH = 9) with 30 mM SDS. Resolution of the most amino acids was achieved with the 15 mM phosphate buffer containing 30 mM SDS. Interestingly,

addition of HP- β -CD was not as effective as what has been observed with o-phthaldialdehyde-derivatized compounds in this buffer [36]. Table S-1 in the Supporting Information summarizes the resulting separations from the optimization of capillary dimensions and buffers.

Once the optimum buffer composition was determined, the pH and concentration of phosphate, and separation temperature and voltage, were optimized. Figure 2A illustrates the effect of buffer pH on the separation of the 19 amino acid mixture. The pH of the buffers tested was 8.0, 8.3, 8.7, and 9.1. A noticeable tailing of the peaks occurred at the lower pH values. We are unsure of the cause of this broadening, but it decreased when the buffer concentration was optimized. While the resolution of the 19 amino acids was similar for most peaks in this pH range, a higher resolution of peak 6 from peaks 5 and 7 was observed at pH 8.3, and as a result, this pH was chosen as the optimum. The phosphate concentration was then optimized with a constant 30 mM SDS concentration. As demonstrated in Figure 2B, 25 mM phosphate yielded the best resolution for peaks 5-7 as well as 16-18 and was chosen as the optimum.

After determining the optimum buffer concentration and pH, the effects of temperature and voltage on resolution were examined. Three separation temperatures were tested: 15, 20, and 25 °C. As can be seen in Figure 2C, at 15 °C, the resolution of peaks 2 and 3 was better than at 20 or 25 °C, while the resolution of the other peaks was worse. The resolution of peaks 9 and 10 was better at 20 °C than at 25 °C, but peaks 7 and 8, 13 and 14, and 16-18 were not resolved. As a consequence, the optimum temperature was determined to be 25 °C. Furthermore, by testing a series of separation voltages ranging from 20 to 29 kV, the best resolution was achieved at 29 kV (current was $\sim 10 \mu\text{A}$).

3.3 Reproducibility, calibration curves, and detection limits

Using the optimized derivatization and separation conditions, the separation of 18 standard amino acids plus D-Nva is shown in blue in Figure 3. Of these analytes, 15 were baseline resolved allowing accurate quantitation. There were two groups of amino acids that were not resolved, which included Thr and Asn in one group and Tyr and Asp in the other. A blank electropherogram that contained D-Nva is shown in red in Figure 3 and it can be seen that it is relatively clean helping to obtain high S/N of the standards.

Three repetitive injections of the derivatized amino acid mixture with D-Nva (1 μM each) were performed and the RSD of migration times were between 0.04 - 0.54% (Table S-2). The intra-day RSD of peak areas were 0.2 - 5.8% after normalization with D-Nva. The normalization did not affect the intra-day peak area RSD ($< 1\%$), but it reduced the inter-day peak area RSD from $\sim 28\%$ to $\sim 12\%$, which allowed a better comparison of the data collected from different experimental days.

Normalized calibration curves were obtained for the 14 amino acids that were baseline resolved, including Ser, Gln, Gly, His, Ala, Glu, GABA, Val, Met, Ile, Leu, Trp, Phe, and Arg, by separating standard solutions with concentrations of 0, 5, 10, 50, 100, 500, 1000, and 2000 nM. Least-squares linear regression indicated good linearity of the plots as the regression coefficients (r^2) ranged from 0.9965 to 0.9999 (Table S-2). The LOD of each

amino acid was determined from the calibration curve and ranged from 0.2 to 7 nM (Table S-2). We attribute the low LODs to the use of NDA as labeling reagent, which produces stable derivatives with high quantum efficiency; optimization of derivatization conditions, which ensured the completion of the reaction and less dilution from the labeling reagent buffers; and optimization of separation conditions with respect to resolution of the amino acids from each other and from background peaks, increasing the S/N for each analyte.

3.4 Amino acids secreted from islets of Langerhans

Once the calibration curves and LODs were determined, separation and quantification of the amino acid secretions from islet samples were attempted. Islets were incubated in BSS with 3 or 20 mM glucose for 1 h and the supernatant was taken and analyzed using the optimized derivatization and separation protocols. Representative electrophoregrams of the separation of amino acids secreted from islets incubated with 3 and 20 mM glucose are shown in Figure 4. As can be seen, the electrophoregrams from the islet samples are similar to those obtained for the standard mixture of amino acids. Hormones secreted from islets, such as insulin or glucagon, were never detected probably due to inefficient derivatization resulting from their large size. Peaks from the islet sample were identified by spiking in amino acid standards. Recoveries were calculated for Gln, Glu, and GABA and were found to be 87 - 106%.

Ser, Gln, Gly, His, Ala, Glu, GABA, Val, Met, Ile, Leu, Trp, Phe, and Arg were all detected in islet secretions, while peaks corresponding to the non-resolved amino acids (Thr or Asn and Tyr or Asp) were also observed. The amounts of each of these released at 3 and 20 mM glucose are summarized in Figure 5 and values are provided in Table S-3. While Glu is thought to be released from α -cells [8], we did not detect a significantly different Glu level from islets incubated in 3 or 20 mM glucose. This result may be due to several reasons, for example, it may be uptaken by the islets during the course of the incubation, or Glu may not be released unless the glucose level is decreased from 20 to 3 mM similar to glucagon release from α - cells. More experiments will be required to understand the different secretion mechanisms of Glu.

Ala and Gln showed a statistically significant increase in release when the glucose concentration was increased to 20 mM. The release of Gly, Val, Met, Leu, Ile, Trp, Phe, Arg, and GABA were statistically lower in the presence of 20 mM glucose compared to 3 mM glucose. While further experiments will need to be performed to delineate the roles that many of these amino acids play in islet physiology, the suppressive effect of glucose on GABA release was in agreement with previous reports using various methods [26-28]. One hypothesis for this suppression is that the high glucose level increases intracellular adenosine triphosphate (ATP) due to glycolysis and oxidative phosphorylation. This increased ATP inhibits the activity of GAD, reducing GABA synthesis [27,28]. To test if our method could be used to investigate the effect of intracellular ATP on GABA secretion, a pharmacological agent, DNP, was added to batches of islets incubated in either 3 or 20 mM glucose. DNP inhibits ATP production in cells by interrupting the proton gradient across mitochondria, which collapses the proton motive force that the cell uses to produce most of its ATP [37]. As illustrated in Figure 5, at 20 mM glucose in the presence of 100 μ M

DNP (green bars), the secretion amounts of Gly, His, GABA, and Arg increased, whereas the level of Glu decreased, compared to 20 mM glucose without DNP (red bars).

When specifically looking at the effect of DNP on GABA (Figure 5 inset), at 20 mM glucose, the amount of GABA released was increased in the presence of DNP as the drug inhibited intracellular ATP production, which removed the inhibition on GAD. These results are similar to another report using HPLC coupled with fluorescence detection to measure the effect of glucose and DNP on GABA secretion [27].

4 Concluding remarks

We have developed an optimized derivatization and separation condition for quantification of multiple amino acids secreted from islets of Langerhans. The optimized method derivatized the amino acids in the high salt buffer used for cellular measurements with little dilution allowing the measurement of 14 amino acids with low LOD. Secretion amounts of amino acids from islets incubated in 3 or 20 mM glucose, with or without the addition of DNP, were successfully quantified. The effect of glucose on GABA secretion was measured and the potential role that ATP plays was tested by the addition of DNP to the incubation buffer.

This method will be useful in delineating the roles that these secreted amino acids play in regulating islet physiology. Even though blood contains many amino acids at high concentrations, the local concentrations of the secreted amino acids could be higher than the blood concentrations allowing them to act in paracrine manner to cells within the islet or to intrapancreatic ganglia. However, further experiments, such as simultaneously measuring amino acid secretion with insulin release, will be required to determine the temporal relationship of the amino acid secretion profiles with respect to hormone release. In addition to islet studies, this method has a great potential for characterizing amino acid secretions from other tissue samples with other biological applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Abbreviations

GABA	gamma-aminobutyric acid
Glu	glutamate
GAD	Glutamate decarboxylase
Gln	glutamine
Ser	serine

Thr	Threonine
Asn	asparagine
Asp	aspartate
Gly	glycine
Ala	alanine
His	Histidine
Tyr	tyrosine
Val	valine
Met	methionine
D-Nva	D-norvaline
Leu	leucine
Ile	isoleucine
Phe	phenylalanine
Trp	tryptophan
Arg	arginine
NDA	naphthalene-2,3- dicarboxaldehyde
HP-β-CD	hydroxypropyl- β -cyclodextrin
BSS	balanced salt solution
DNP	2,4-dinitrophenol
ATP	adenosine triphosphate
RFU	relative fluorescence units
HPLC	high-performance liquid chromatography
CE	capillary electrophoresis
MEKC	micellar electrokinetic chromatography

5 References

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Figure 1. Optimization of derivatization conditions

(A) Derivatization times were tested by mixing a solution containing 1 μ M each of Ser, Gln, Glu, GABA, Met, Ile, Leu, and D-Nva with 5 mM NDA and 20 mM cyanide and allowing the reaction to proceed for 5, 10, 20, 60, and 120 min. The average peak areas from the resulting peaks in the electropherogram are plotted versus the derivatization time. Each time period was performed in triplicate and the mean value is plotted with error bars corresponding to ± 1 standard deviation. (B) The effect of the derivatization temperatures on the resulting peak areas is shown using the same reaction conditions as in (A) with a 20 min derivatization time. Each temperature point was performed in triplicate and the average peak area is shown with error bars corresponding to ± 1 standard deviation.

Figure 2. Optimization of separation conditions

In all electropherograms, a 19 amino acid mixture was separated using 29 kV and the peaks are numbered as the following: 1 = Ser, 2 = Thr, 3 = Asn, 4 = Gln, 5 = Gly, 6 = Ala, 7 = His, 8 = Glu, 9 = Tyr, 10 = Asp, 11 = GABA, 12 = Val, 13 = Met, 14 = D-Nva, 15 = Ile, 16 = Leu, 17 = Trp, 18 = Phe, and 19 = Arg. **(A)** The separation of these 19 amino acids (1 μ M each prior to derivatization) with a separation buffer of 15 mM phosphate, 30 mM SDS, at pH 8.0, 8.3, 8.7, and 9.1 are shown. **(B)** The effect of phosphate concentration was examined by using 15, 20, 25, and 30 mM phosphate. All buffers contained 30 mM SDS at pH 8.3. **(C)** Using 25 mM phosphate at pH 8.3 with 30 mM SDS, separation temperatures of 15, 20, and 25 °C were examined. All electropherograms are plotted with relative fluorescence units (RFU) on the y-axis and the electropherograms are offset for clarity.

Figure 3. Optimized electropherograms of standard amino acid mixtures

The blue electropherogram (top trace) was obtained after derivatization of a 1 μ M amino acid mixture using the optimized conditions. Peak identities are the same as described in Figure 2. The red electropherogram (bottom trace) was obtained when a background BSS solution containing D-Nva was derivatized. In both cases, optimized separation conditions were used, which were a 25 mM phosphate separation buffer, pH 8.3, containing 30 mM SDS with a 29 kV separation voltage and a separation temperature of 25 $^{\circ}$ C.

Figure 4. Representative electropherograms of islet secretions

Secretions from 25 islets incubated in 3 or 20 mM glucose were derivatized and separated according to the optimized procedures. The blue (bottom) and red (top) traces were the electropherograms obtained when islets were incubated in 3 and 20 mM glucose, respectively. Peak identities are the same as described in Figure 2.

Figure 5. Effect of glucose and DNP on secretion amounts

The secretory amounts of the 14 baseline resolved amino acids are summarized. Amounts measured after 1 hour incubation in 3 mM glucose are shown in black, in 20 mM glucose in red, and in 20 mM glucose with DNP in green. The average of three replicate trials is shown with error bars corresponding to ± 1 S.E.M. Significance was determined using a one-tailed t-test with: (*) = $p < 0.05$ compare to 3 mM glucose, or (**) = $p < 0.05$ compared to 20 mM glucose without DNP. Inset shows a zoomed in view of the amount of GABA detected at the indicated conditions.