AMUZA
Case Study

Studying Pancreatic Islet Cell Biology and Its Implication in Diabetes with HPLC-ECD

In the realm of diabetes research, the intricate interplay of pancreatic islet cell biology holds a crucial key to understanding the pathophysiology of both Type I and Type II diabetes. Among these cells, human pancreatic beta cells stand out not only for their pivotal role in insulin production but also for their unique possession of gamma-aminobutyric acid (GABA) – a neurotransmitter conventionally associated with neuronal function. However, recent studies have unveiled a fascinating aspect of beta cell biology: the presence of a substantial amount of GABA within their cytosol, actively secreted into the extracellular milieu.

At the heart of this endeavor lies the enigmatic nature of GABA's signaling mechanisms within the islet microenvironment. While it is established that GABA serves as an autocrine communicator within beta cells, triggering signaling cascades that modulate glucagon secretion from neighboring alpha and delta cells, the full extent of its impact remains elusive.

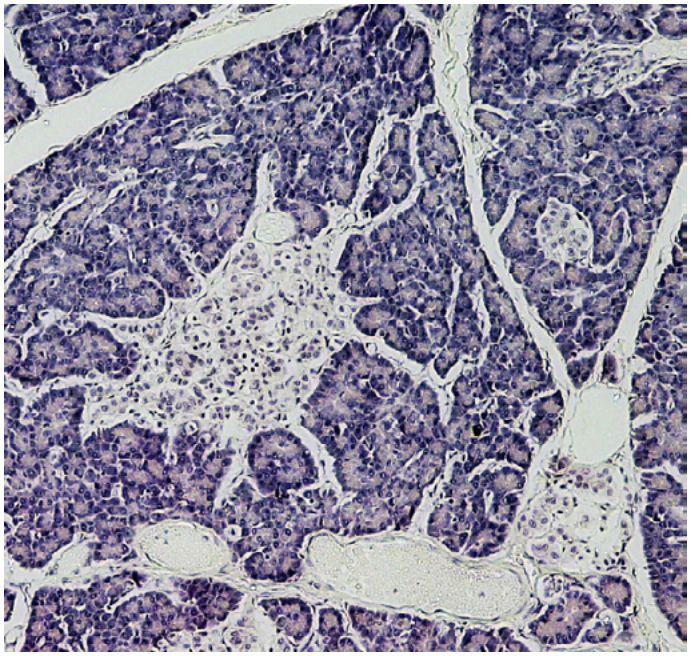
Therefore it is important to elucidate the network of interactions orchestrated by secreted GABA and its influence on pancreatic islet cell biology. Such elucidation holds profound implications for the development of therapeutic interventions targeting aberrations in pancreatic islet cell function, offering a potential avenue for combating diabetes.

Achieving this ambitious objective is not without its challenges, as measuring GABA accurately can be elusive. One approach is to use High-Performance Liquid Chromatography coupled with Electrochemical Detection (HPLC-ECD) as a powerful and sensitive analytical tool. Allowing for characterization of GABA dynamics within the complex milieu of pancreatic islet cells, facilitating a comprehensive understanding of its role in diabetes pathogenesis.

Here we highlight the efforts of a researcher at the University of Florida using HPLC-ECD to shed light on the intricate nature of GABA signaling in pancreatic islet cell biology. Through careful experimentation and analysis, they endeavor to pave the way towards innovative strategies for diabetes management, leveraging the potential of GABA as a protective agent against metabolic dysfunction. Our interview with them has been edited for formatting.

Anonymous Ph.D. Candidate at the University of Florida

Research Background



Amuza: What is the goal of your research? What social problem do you want to tackle?

Researcher: We study human pancreas beta cells, which produce insulin, outside of neurons, beta cells are the only other cell types that have GABA. They have a large amount of GABA in the cytosol, and actively secret GABA. The importance of studying this is that it has implications for Type I and Type II diabetes. In diabetic patients, GABA is diminished in these islet cells. What we do know is that GABA is an autocrine communicator and can signal alpha and delta cells to stimulate the glucagon secretion pathway. We do not have much further understanding than that. The goal is to determine other roles of secreted GABA and how this affects pancreatic islet cell biology, and how this relates to diabetes. In theory, the glucagon secretion pathway is a potential point of therapeutic intervention for diabetes and GABA may be protective.

Amuza: What are some of the biggest challenges you face during your research journey?

Researcher: First thing is that GABA is really hard to immunostain for, there are some decent antibodies but it can still be challenging. GABA concentration is also challenging, secreted GABA concentrations are low so sensitive methods of detection are needed. HPLC-ECD is very helpful in detecting low concentrations of GABA, with some experimental tweaking.

Prior HPLC Experience

Amuza: What was your experience with HPLC before the purchase of the HTEC?

Researcher: I've had some experience using other systems. In the past I did some work within the pharmaceutical industry where we were looking at size and purity of compounds. It was a larger version of the HTEC, but I do not recall the manufacturer of the system.

Amuza: Did you have any other options to measure neurotransmitters within your samples? If yes, please let me know which and why you chose the HTEC?

Researcher: Prior to HPLC we tried multiple GABA ELISAs and really struggled with accurate detection as GABA is not a protein and the sensitivity of the ELISA kits were not optimal.

User Experience

Amuza: What benefits and features stood out to you during your research experience with the HTEC?

Researcher: One of the main benefits is that the HTEC is fairly easy to do minor maintenance on such as changing pump pistons, diaphragms, electrodes, and such. Additionally, having the autosampler is very nice as it works well with the HTEC, I'm not sure why people wouldn't purchase this as this increases the throughput significantly.

Amuza: What type of samples do you run and why are they important?

Researcher: We typically run cell culture supernatant, and occasionally cell lysate since we are looking at GABA secretion and production. The lysate is important because we can explore GABA secretion as a function of glutamate levels. We are using shRNA techniques to either inhibit or increase different protein expression and see how GABA secretion and insulin production are affected.

Amuza: How was your experience using the HTEC different from previous methods you have used for neurotransmitter analysis?

Researcher: The HTEC is very straightforward to use as long as you are diligent with the maintenance of the system, once I figured out the best practices for maintenance I have had very little issues. Additionally, the sensitivity for GABA is far better than any other method we have tried including biosensors, immunofluorescence, and ELISAs.

Amuza: How likely are you to recommend the HTEC to a colleague?

Researcher: If they are looking for consistent detection of neurotransmitters I would definitely recommend it, I would rate it around a 6 or 7 out of 10. I think maybe the price is one thing that could be of concern for some labs, depending how frequently it's used.

Amuza: How has the HTEC improved your research? Has it helped you solve any difficult challenges you were facing in your research? Please let us know the details.

Researcher: It's given us the sensitivity needed to analyze more functional data, doing temporal studies and looking at the effect of inhibitors or activators of multiple different factors such as protein expression and how this affects GABA secretion.

Amuza: Do you have any suggestions for improvements you would like to see in the future?

Researcher: While I realize some of these requests would be difficult to satisfy, I wish the equilibration of the separation column to the mobile phase did not take so long, running overnight is not ideal. Another wish list item would be increased sensitivity and more software based hardware diagnosis, such as pump malfunctions, piston may be misaligned, etc. Ideally, I would like to see more transparency on the lifetime of consumables for the system, like how many injections can a column work for, etc. Also having some more in-depth instruction in the manuals on replacing these consumable parts would be nice.

Amuza Comment: Our new HTEC-600 system features a new pump design making diagnosis and troubleshooting of issues including general maintenance significantly easier. We are actively improving our support pages which includes the addition of video content to demonstrate maintenance and best practice procedures.

Future Application

Amuza: Do you have any planned future applications of the HTEC? If not, what do you imagine you would do with it once this project is complete?

Researcher: I don't think we really have anything major on the radar right now. One reason being, we are concerned with switching between applications. I occasionally process GABA, glutamate and taurine samples from other lab members to assist with their projects.