CALCIUM SIGNALS IN RAT PANCREATIC INS-1 BETA CELLS

The pancreas provides digestive enzymes for the small intestine and hormones that regulate blood glucose levels in the body. The two pancreatic endocrine hormones, insulin and glucagon, produced by the pancreatic islet cells, are responsible for maintaining the blood glucose levels in the body. Like all endocrine cells, insulin is a protein that binds with cells (eg, muscle, liver, and adipose tissue). The cells have receptor proteins to receive the signals from hormones such as insulin. Some receptor proteins signal to pore-forming proteins, called ion channels. These ion channels allow the entry and exit of specific ions into and out of cells. We hypothesized that rat pancreatic insulinoma-1 (INS-1) beta cells express two ion channels, the transient receptor potential vanilloid subfamily 1 (TRPV1) and the transient receptor potential ankyrin subfamily 1 (TRPA1), and that they are both in the plasma membrane of an INS-1 cell and in the membrane of its endoplasmic reticulum, functioning as both calcium influx and calcium release channels. The method we used to test this hypothesis was fluorescent calcium imaging. We incubated INS-1 cells in a calcium-sensitive dye called Fura-2 AM for 45 minutes at a time. Fura-2 changes the light it emits when calcium binds to it. A calcium-imaging set-up measured these wavelength changes. The resulting calcium concentration was displayed and saved on a computer. For TRPV1, we used capsaicin and anandamide, substances predicted to activate TRPV1, as test compounds.[1] For TRPA1, we used icilin, a substance predicted to activate TRPA1, as a test compound.[2,3] We also measured inward and outward currents of cells using the whole-cell patch clamp technique in order to determine whether it really was TRPV1 and TRPA1 that caused the resulting calcium signals.[4] We concluded that the calcium channels TRPV1 and TRPA1 are expressed in INS-1 cells and therefore may contribute to insulin secretion.

Student Researcher: Marifel Barbasa, James Campbell High School
Mentor: Andrea Fleig, University of Hawaii at Manoa and The Queen's Medical Center, Honolulu, Hawaii

BACKGROUND

Calcium is an important second messenger involved in muscle contraction, hormone release, and nerve cell signalling. A cell has two calcium sources: extracellular calcium, which can be obtained through calcium influx from outside the cell; and intracellular calcium, which can be obtained through calcium release from the endoplasmic reticulum, an organelle within the cell. With calcium influx, calcium can enter the cell if, for example, an agonist binds to calcium-conducting ion channel in the plasma membrane of the cell, thereby activating the channel and causing it to allow calcium to enter the cell.

On the other hand, ion channels within the membrane of the endoplasmic reticulum permit calcium release into the cytosol. A classical pathway of calcium release is when an agonist from outside the cell binds to a receptor protein within the plasma membrane of the cell, triggering the G-protein to activate the phospholipase C (PLC), a protein that makes inositol triphosphate (IP_3). The IP_3 then travels to and binds with the IP_3 receptor within the membrane of the endoplasmic reticulum, causing it to open and allow calcium release into the cytosol.[5] In this study, we focused on the ion channel mechanism and not on the receptor pathway of calcium release.

TRPV1 and TRPA1 belong to the superfamily of transient receptor potential ion channels (Niforatos).[6] TRPV1 is specifically activated by capsaicin and anandamide.[1] Capsaicin is the hot ingredient in a chili pepper, and anandamide is a naturally occurring hormone in the human body. TRPA1 is specifically activated by icilin, which is a super-coolant originally developed for toothpaste and nasal spray.[2,3] TRPV1 has been shown to be expressed in the pancreatic rat insulinoma (RIN) beta cell line, but expression of TRPA1 in beta cells is currently unknown. The RIN cell line INS-1 is also an insulin secreting beta cell. Since insulin secretion critically depends on cellular calcium signaling, expression of TRPV1 or TRPA1 in these cells may contribute to insulin secretion.[7]

We hypothesized that INS-1 beta cells from the pancreas of a rat contain two calcium ion channels, identified as TRPV1 and TRPA1. Our second hypothesis was that both TRPV1 and TRPA1 are functional as calcium influx and calcium release channels, and are located in the plasma membrane of a cell and in the membrane of its endoplasmic reticulum.

METHODS

We used the calcium-imaging technique and the whole-cell patch clamp technique. With the calcium-imaging technique, an agonist is applied to an intact cell, which has been previously loaded with Fura-2. Fura-2 is a calcium-sensitive dye that changes its wavelengths in response to the amount of calcium bound to it. The range of calcium that Fura-2 can measure accurately is from 20 nm calcium to 2 μm calcium. The change in its emission light intensity assessed at 360 nm and 380 nm excitation wavelengths are projected onto a computer screen, and from this, specialized computer software calculates the amount of calcium a cell currently holds. With the patch clamp technique, two glass pipettes are used, and Fura-2 is no longer needed. One

pipette is used as an application while the other pipette is used to measure currents. Using a three-step process with the patch pipette, the pipette forms a tight seal onto the cell membrane, the membrane patch underneath is disrupted such that intracellular solution now enters the cell, and ionic fluxes going into and out of a cell are measured as current in amperes.

RESULTS

Capsaicin causes Ca signals in INS-1

We first wanted to know whether capsaicin, the agonist in this case, would alter the calcium concentrations inside of a cell, leading us to use the calcium-imaging technique. Thus, in the presence of a 1 mM calcium extracellular solution (1 mM Ca-Na ringer: 1 mM $CaCl_2$ + 140 mM NaCl + 2.8 mM KCl + 2 mM $MgCl_2$ + 10 mM Hepes-NaOH + 10 mM glucose), capsaicin was applied to intact cells loaded with Fura-2. The capsaicin application was a mixture of capsaicin and the extracellular solution. Overall, the calcium concentration began with a measurement of 50 nm, and once capsaicin was applied, this concentration increased. After capsaicin application was stopped, calcium levels decreased. In conclusion, capsaicin does cause calcium signals in INS-1 cells. Therefore, our next question was: where were these signals coming from? Were they signals due to calcium influx, calcium release, or both?

In the next step, we applied capsaicin in an extracellular solution devoid of any calcium to remove the possibility of calcium entering the cell from the outside (0 Ca-Na ringer: 140 mM NaCl + 2.8 mM KCl + 2 mM $MgCl_2$ + 10 mM Hepes-NaOH + 10 mM glucose). Calcium concentration still increased during the time of application and lowered again after application was stopped. Therefore, capsaicin caused calcium signals in the rat pancreatic INS-1 beta cell line, due to calcium release from the endoplasmic reticulum. Our next query was, is the capsaicin response due to TRPV1, and is TRPV1 also located in the plasma membrane?

Capsaicin causes TRPV1 channel activity

To this end we needed to measure capsaicin-induced currents, for which we used the patch clamp technique. Once again we bathed the cells in 1 mM Ca-Na ringer. The internal solution applied to each cell with the patch pipette was Cs-glutamate ringer (120 mM Cs-glutamate + 8 mM NaCl + 1 mM $MgCl_2$ + 10 mM Hepes-CsOH + 10 mM Cs-BAPTA). We used the following voltage protocol: the protocol started at 0 mV, lowered to −100 mV, ramped up to +100 mV, and then lowered to 0 mV. The voltage protocol was measured over a period of 50 ms at 2-second intervals. For analysis, the negative currents were the inward currents of a cell, measured at −80 mV, while the positive currents were the outward currents, measured at +80 mV. Our analysis of the average current development indicated that a sharp increase in both inward currents and outward currents occurred in response to capsaicin application. After application was stopped, the currents decreased. Therefore, we can conclude that it was TRPV1 that had caused the calcium signals during the calcium-imaging process, meaning TRPV1 is expressed in rat pancreatic INS-1 beta cells, and is functional both in the plasma membrane and the ER.

Icilin causes Ca signals in INS-1

We next asked the question whether TRPA1 is expressed in INS-1 cells. The same experimental approach as for capsaicin was used. When applying icilin to intact cells loaded with Fura-2 in the presence of 1 mM calcium in the extracellular solution, we could again observe calcium signals.The calcium oscillated back down to its original concentration after application was stopped. Using icilin as an application, we posed the same question we had when using capsaicin; were those calcium signals caused by calcium influx, calcium release, or both? So the icilin experiment above was repeated, but this time, the icilin application did not contain calcium. We found a clear calcium signal. Therefore, icilin caused calcium signals in the rat pancreatic INS-1 beta cell line due to calcium release from the endoplasmic reticulum. Another step had to be completed to determine whether it really was TRPA1 that caused those calcium signals and whether TRPA1 is also located in the plasma membrane.

Icilin causes TRPA1 channel activity

Using the patch clamp technique, the process here was the same as the process used for the capsaicin application. The currents measured were at 50 seconds, just when icilin application began, and were lower currents than those measured during the icilin application. We found that both the inward and outward currents of the cells increased during icilin application and then gradually decreased after icilin application was stopped. There was more significant increase in inward currents as compared to outward currents. We therefore conclude that icilin causes TRPA1 channel activity, indicating that it was TRPA1 that caused the calcium signals during the calcium-imaging process. Thus, TRPA1 is functional as a calcium influx channel and is located in the plasma membrane of INS-1 cells.

CONCLUSIONS AND OUTLOOK

In conclusion, we can state that rat pancreatic INS-1 beta cells contain two calcium ion channels, TRPV1 and TRPA1. We can also infer from our data that both channels are functional as calcium influx and calcium release channels, and are located in the plasma

membrane of a cell and in the membrane of its endoplasmic reticulum. In the future, we will test whether the substance anandamide, a naturally occurring hormone in the human body, activates TRPV1 in INS-1 cells. Furthermore, we will test whether mustard seed oil activates TRPA1 in INS-1 cells with a better response than what icilin has presented to us.[8]

ACKNOWLEDGMENTS

The author would like to thank, Dr. Andrea Fleig, Dr. George Hui, Ms. Kae Myriam Pusic and the National Institutes of Health and the National Institute of Diabetes and Digestive & Kidney Diseases.

REFERENCES

1. Morita A, Iwasaki Y, Kobata K, et al. Lipophilicity of capsaicinoids and capsinoids influences the multiple activation process of rat TRPV1. *Life Sci*. 2006;79:2303–2310.
2. Chuang HH, Neuhausser WM, Julius D. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron*. 2004;43:859–869.
3. Sawada Y, Hosokawa H, Hori A, Matsumura K, Kobayashi S. Cold sensitivity of recombinant TRPA1 channels. *Brain Res*. 2007;1160:39–46.
4. Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. *Molecular Biology of the Cell*. 2nd ed. New York: Garland Publishing, Inc; 1989.
5. Shah PK, Sowdhamini R. Structural understanding of the transmembrane domains of inositol triphosphate receptors and ryanodine receptors towards calcium channeling. *Protein Engineering*. 2001;14:867–874.
6. Niforatos W, Zhang XF, Lake MR, et al. Activation of TRPA1 channels by the fatty acid amide hydrolase inhibitor 3′-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597). *Mol Pharmacol*. 2007;71:1209–1216.
7. Akiba Y, Kato S, Katsube K, et al. Transient receptor potential vanilloid subfamily 1 expressed in pancreatic islet beta cells modulates insulin secretion in rats. *Biochem Biophys Res Commun*. 2004;321:219–225.
8. Zurborg S, Yurgionas B, Jira JA, Caspani O, Heppenstall PA. Direct activation of the ion channel TRPA1 by Ca2+. *Nat Neurosci*. 2007;10:277–279.