Effect of Insulin on Mast Cell Physiology

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Diabetic patients have been reported to be less prone to allergic reactions. This is thought to be due to a reduced number of mast cells, an immune cell type, and a reduced responsiveness of these cells to release histamine. In mast cells, histamine release is linked to intracellular calcium signalling. Calcium can enter the mast cell through specialized pore-forming ion channels in the plasma membrane. The amount of calcium entering the cell is also determined by the activity of other ion channels, including potassium channels. It seems that blood insulin levels determine mast cell number and their responsiveness. We hypothesized that insulin furthers the growth of the mast cell model RBL2H3. Our second hypothesis is that insulin alters the activity of the potassium channel in these cells. We studied these hypotheses using tissue culture and whole-cell patch clamp technique.

Background

Type 1 diabetes mellitus is caused by the destruction of pancreatic beta cells, which leads to a deficiency in glucose-induced blood plasma insulin levels. Patients with type 1 diabetes are less prone to allergic reactions[1] because mast cells play a central role in the onset of allergic reactions.[1] Patients with type 1 diabetes have fewer mast cells to respond to allergens. Furthermore, these mast cells are less responsive to stimuli that cause histamine release.[2] Histamine binds to the histamine receptor on target cells, which can lead to, for example, smooth muscle contraction, relaxation, hives, a running nose (allergic rhinitis) and other allergic symptoms. Research indicates that insulin is partly responsible for maintaining healthy mast cell populations.[3]

Calcium is an essential nutrient used by many physiological functions, including healthy bones, teeth, skin and muscle. Histamine release in mast cells is controlled by calcium levels inside the cell. Calcium can enter the cell through specific pore-forming proteins in the plasma membrane, so-called calcium ion channels. Mast cells express at least two types of calcium channels: the CRAC channel (calcium-release activated calcium) and MagNuM channel (magnesium-nucleotide regulated metal). Other ion channels, such as the potassium channel, regulate the amount of calcium that can enter the cell.

The current research project investigated the effect of insulin on mast cell growth and ion channel expression levels of potassium channels in the RBL2H3 mast cell model. The goal was to see whether insulin could increase or decrease the growth of RBL2H3 cells and whether this could be related to the relative expression levels of potassium ion channels. If expression levels were altered, this would indicate that the cell's calcium homeostasis could be altered as well. We hypothesized that insulin furthers the growth of the mast cell model RBL2H3. Our second hypothesis is that insulin alters the activity of the potassium channel expressed in RBL2H3 cells.

Methods and Materials

We used tissue culture techniques and the whole-cell patch clamp technique. With the tissue culture technique, 500,000 cells were inoculated in 15 mL of tissue culture media in the presence or absence of 100 nM insulin. 100 nM was chosen to reflect physiological insulin levels in the body. Cells were harvested and counted 24 hours after incubation start using a hematocrit. This experiment was repeated four times, the numbers averaged and plotted in a graph. The statistical analysis was done using the Student's *t* test.

The patch-clamp technique is a three-step process lowering a glass patch pipette filled with a potassium-based solution toward the cell. The pipette then forms a tight seal with the cell membrane and the membrane patch underneath the pipette opening (1 μM diameter) is disrupted such that intracellular solution now enters the cell. This allows the measurement of ionic fluxes going into and out of a cell as current in amperes. For experiments, cells were or were not exposed to 100 nM insulin for 24 hours before patch clamping. Potassium currents were measured by applying voltage ramps spanning from −100 mV to +100 mV over 50 ms and acquired

every 2 s. The potassium current size was measured at –80 mV and 100 s into the whole-cell experiment. Data were averaged and statistically analysed using the Student's *t* test.

RESULTS

Our results indicate that the addition of 100 nM insulin to the growth media furthers mast cell growth as evidenced by the rat RBL2H3 mast cell model. The cell number of cells exposed to insulin was double that of the control after 24 hours of incubation (56,000 cells/mL ± 11,400 vs 100,125 cells/mL ± 15,300, $n = 4$ each). This not only indicates that RBL2H3 cells grow faster in the presence of insulin, it also indicates that these cells must express the insulin receptor in their plasma membrane.

When probing RBL2H3 cells for insulin-induced changes in potassium channel expression, we found that incubation of cells with 100 nM insulin for 24 hours significantly reduced the measured potassium currents by about 50%. The current amplitude measured in control cells at −80 mV was −134 pA ± 18 pA ($n = 35$) and in cells exposed to insulin the current amplitude was −62 pA ± 18 pA (n = 35). This indicates that the potassium current expressed in mast cells may be involved in insulin-induced down-regulation of histamine release or the stunt of mast cell growth as observed in diabetic patients.

CONCLUSIONS

In conclusion, we can state that the rat mast cell line RBL2H3 grows abundantly in the presence of insulin. This indicates that these cells have insulin receptors expressed in their plasma membrane and these receptors support cell growth. The reduced amplitude of potassium currents in the presence of insulin needs to be evaluated further in the light of insulin effects on other ion channel species in this cell. Therefore, the absence of insulin in diabetic patients will blunt healthy mast cell growth.

Future research should investigate whether the calcium influx ion channels in RBL2H3 cells, CRAC and MagNuM, are altered in the presence of insulin.

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REFERENCES

1. Carvalho VF, Barreto EO, Cordeiro RS, Lagente V, Martins MA, Silva PM. Mast cell changes in experimental diabetes: focus on attenuation of allergic events. *Mem Inst Oswaldo Cruz*. 2005;100 Suppl 1:121–125.
2. Kalichman MW, Powell HC, Calcutt NA, Mizisin AP. Mast cell degranulation and blood-nerve barrier permeability in rat sciatic nerve after 7 days of hyperglycemia. *Am J Physiol*. 1995;268:H740–H748.
3. Lessmann E, Grochowy G, Weingarten L, et al. Insulin and insulin-like growth factor-1 promote mast cell survival via activation of the phosphatidylinositol-3-kinase pathway. *Exp Hematol*. 2006;34:1532–1541.