MOLECULAR MECHANISMS OF THE REGULATION OF THE FUNCTION OF PANCREATIC B CELLS

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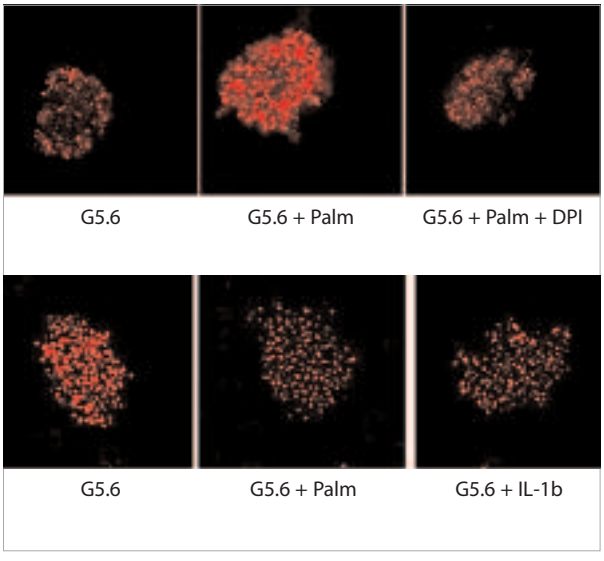

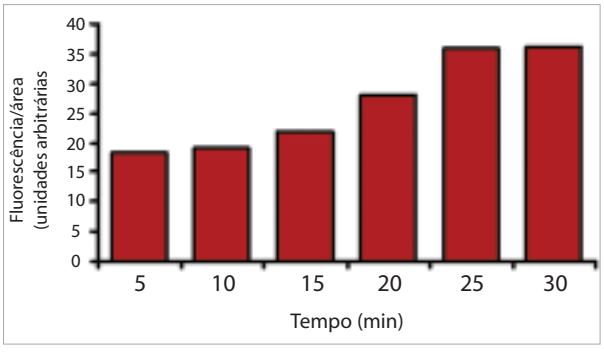

Effect of palmitate or IL-1b on ROS production assessed by ethidium fluorescence in isolated pancreatic islets. The production of ROS by isolated rat pancreatic islets was determined by using a hydroethidine oxidation assay. The fluorescence intensity of islets was analyzed by Zeiss confocal microscopy

We first demonstrated that pancreatic B cells express NAD(P)H oxidase, and that this enzyme seems to be involved in superoxide generation during the process of insulin secretion. This study started after our observation that glucose controls antioxidant enzyme activities such as superoxide dismutase (SOD), catalase and glutathione peroxidase. We found that the increase in SOD activity is directly correlated with increasing glucose concentration. Considering that SOD activity increases concomitantly with raises in superoxide generation in several tissues, we examined the same phenomenon in pancreatic B cells. We found not only a direct correlation between the increase of glucose concentration and superoxide generation by isolated rat islets, but also that the production of these compounds was dependent on NAD(P)H. Unpublished results from our laboratory indicate that glucose, palmitate and interleukin-1B increase superoxide generation through NAD(P)H oxidase activity. These compounds also increase the expression of some NAD(P)H oxidase components. The function of NAD(P)H oxidase in the process of insulin secretion and the action of other endogenous substances on the activity of this enzyme were then investigated. Isolated pancreatic islets chronically exposed to high glucose concentration, free fatty acids, or interleukins have shown impaired insulin secretion. These changes are, at least in part, due to the production of reactive oxygen species. We also investigated whether NAD(P)H oxidase could be involved in these process as well. This project will initially study the participation of NAD(P)H oxidase in the molecular mechanisms that regulate the glucose – and palmitate-induced insulin secretion. Among the objectives of this project are the study and the evaluation of the effect of oxidative stress in RINm5F in order to clarify the participation of this enzyme in the impairment of secretory events in pancreatic B cells.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

We are evaluating the role of NAD(P)H oxidase in the regulation of the secretion of insulin in isolated islets. We demonstrated that NAD(P)H oxidase is modulated by glucose, palmitate and interleukin -1β which increased the proteic expression of the subunit $p47^{PHOX}$ and the production of superoxide. Hydrogen peroxide (H_2O_2) formed from the superoxide decreased in the presence of high concentration of glucose due to the activation of the pentose path, an important metabolic path responsible largely for the maintenance of the antioxidant activity of the B cells of the pancreatic islet. The palmitate induced the activation of the NAD(P)H oxidase through the membrane receptor GPR40 and/or through its metabolization. Melatonin decreased the production of ROS by pancreatic islets without promoting alteration in the subunits of the NAD(P)H oxidase thus suggesting that the hormone acts by different routes stimulating antioxidant enzymes. In another series of experiments, palmitate and glucose interacted, and altered the expression of some early genes (*C-fos* and *C-jun*) and proteins of the mitogenic paths (ERK1/2, AKT and SAPK/JNK), which could also be related to long term alterations in the secretion of insulin. Oleate in turn increased the expression of insulin induced by 5.6 e 16.7mM of glucose without altering the proteic expression of the *Pkc* and $Gp91^{PHOX}$. In the presence of 16.7 mM of glucose, oleate increased the expression of GPR40, although its expression and the expression the pro-insulin had decreased. Exact like the palmitate, oleate may be acting through its own metabolization and/or via GPR40. Mice fed with a diet rich in medium-chain fatty acids present peripheral resistance to insulin through isolated islets, which however decrease the expression of the hormone and increase the cell death percentage.

Our research has brought important discoveries related to the oxidative stress involved in the initial phases of the onset of Diabetes mellitus.

MAIN PUBLICATIONS

As the project has just commenced, data obtained will lead to publications on due course.

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