INSULIN - INDUCED MEMBRANE AREA INCREASE IN SINGLE RAT ADIPOCYTES

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Introduction. Adipocytes play a key role in energy balance by serving as a major site of storage and as an endocrine organ, secreting different hormones and cytokines that regulate energy storage and metabolism in other tissues as well (8). Along with skeletal muscle cells adipocytes are the main target for insulin action to lower blood glucose level. Insulin increases glucose permeability of adipocytes by stimulating the incorporation of glucose transporters into the plasma membrane by exocytosis (7). Exocytosis and endocytosis are ubiquitous processes of eukaryotic cells. Exocytosis of vesicles is associated with an increase, while the process of endocytosis causes a decrease in the plasma membrane surface area, respectively.

Methods. Net changes in plasma membrane surface area can be monitored directly by the electrophysiological measurements of membrane capacitance ($C_m$), a parameter linearly proportional to the plasma membrane area (5). We employed the whole-cell patch-clamp technique to measure the membrane capacitance of single white rat adipocytes. During experiments we added insulin to a final concentration of 1 μM.

Using confocal microscopy and the styryl fluorescent dye FM1-43, a membrane area marker (3) we monitored cumulative exocytosis in single adipocytes. Cells were placed in a recording chamber, the recording medium contained 5 μM FM1-43. During experiments we added insulin. Upon vesicle fusion membrane area increases, hence the FM1-43 fluorescence intensity increases due to staining of freshly fused membrane (6). The time-dependent changes of FM1-43 fluorescence intensity of a 2-μm thick rim of the plasma membrane area were determined.

Results and discussion. The application of insulin resulted in a significant increase in $C_m$ (12 %) compare to control cells (2 %), indicating a dominating role of exocytosis over endocytosis. Insulin-stimulated increase in $C_m$ could be either due to a number of processes. These include: the stimulation of exocytosis, the inhibition of endocytosis, or the combination of both processes. To further study this question, we compared $C_m$ recordings with the fluorescence intensity measurement of FM1-43 stained plasma membrane, which reports cumulative exocytosis (2, 3).

Following the application of insulin, fluorescence intensity of FM1-43 stained membrane increased significantly due to the incorporation of new membrane into the plasma membrane by exocytosis. At rest, FM1-43 fluorescent intensity increased on average by 0.92 %/min, which was significantly higher than the rate of increase in $C_m$ at rest (0.15 %/min). The estimated rate of endocytosis at rest is therefore around 0.8 %/min. These results indicate that at rest the rates of exocytosis and endocytosis are well balanced. After insulin application the rate of FM1-43 fluorescent intensity rate increase was significantly higher with 3.7 %/min than the rate of $C_m$ increase of 2.7 %/min. The estimated rate of endocytosis after insulin application is around 1%/min. These results indicate that insulin stimulates mainly if not only exocytosis. However, stimulation of the rate of endocytosis is also present in a small extent, but whether this is significant can not be concluded from these results.
Figure 1: FM1-43-stained adipocyte. Confocal micrograph of FM1-43-stained adipocyte before (left panel) and at the end of recording after the insulin application (+ insulin; right panel). The fluorescence intensity is proportional to the surface area of the plasma membrane. Bar indicates 10 μm.

Our results show directly that in adipocytes insulin increases membrane area, which is likely due to an increased rate of exocytosis that exceeds the insulin-induced increase in membrane retrieval by endocytosis. The enhanced rate of exocytosis is probably associated with transporter density regulation (4) and/or secretion (1).