

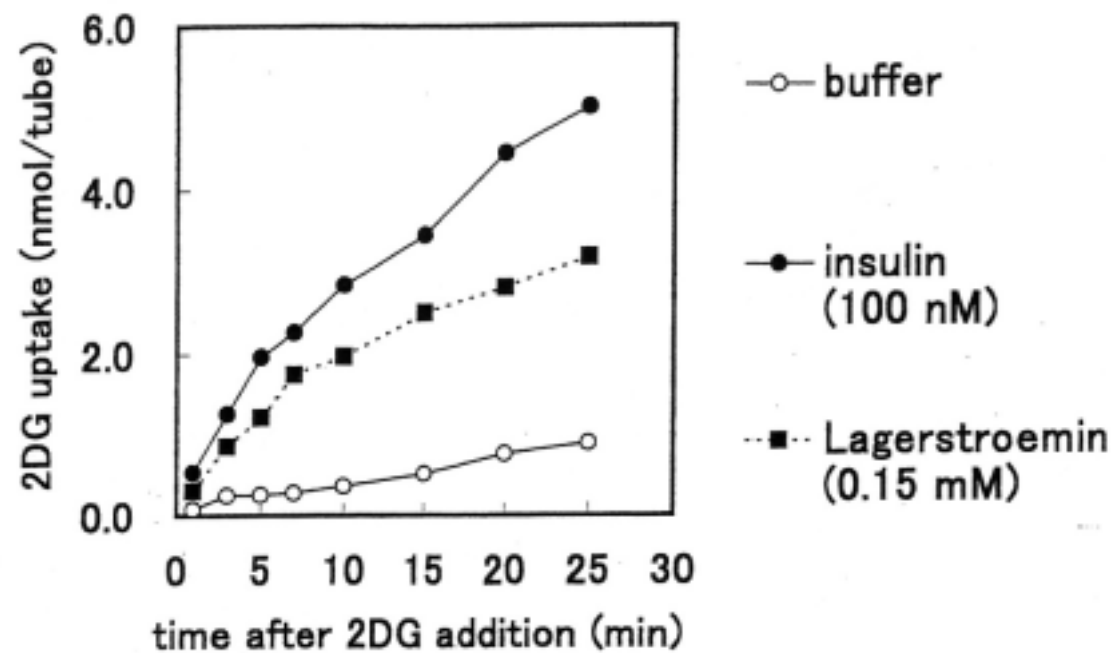
Preface

Banaba (*Lagerstroemia speciosa*) is deciduous tree, which is distributed in Southeast Asia. In the Philippines, it is used as herbal medicine for diabetes mellitus. We report that an ellagitannin contained in banaba leaves called Lagerstroemin showed insulin-like effect in rat adipocytes.

Banaba leaves contain many varieties of tannins. We examined glucose uptake enhancement activity of some of these tannins. Among the tannins, we indicated four kinds of tannins here, 2 showed the activity and the other two did not.

As the result of the examination, it was confirmed that Lagerstroemin has a strong activity, which is approximately 60% of the maximum activity of insulin. Casuarinin also has an activity though it is weak. However, other tannins including Lagerstannin B and Hippophaenin A did not show a remarkable activity.

We examined glucose uptake enhancement activity of structure related compounds of Lagerstroemin. Ellagic acid, which is a partial structure of Lagerstroemin, did not show any activity. Because Pedunculagin, a kind of ellagitannin, showed glucose uptake enhancement activity (data not shown), we also examined a series of gallo tannins, which are tannin analogues. As a result, only 1, 2, 3, 4, 6 - penta galloyl glucose showed very weak activity. From the results showed in Figure 2 and Figure 3, we started elucidating the mechanism of Lagerstroemin which has stronger glucose uptake enhancement activity than the other tannins contained in banaba leaves.



We treated epididymal adipose tissues of 5-6-week-old male wistar rats with collagenase and pre-treated free adipocytes. After incubating the adipocytes with insulin (100nM) or Lagerstroemin (0.15mM) at 37 for 20 minutes, we added 2-[³H] deoxyglucose (2DG) and measured the uptake amount for the period of time shown in the figure.

Figure 1. Insulin-like activity of Lagerstroemin (1) (Glucose uptake enhancement activity)

In the rat adipocytes, both of insulin and Lagerstroemin helped to take more 2DG into the cells depending on uptake time after 2DG addition. Up until 5 minutes, the uptake amount increased linearly. Therefore, in the following experiments, we decided the uptake time 5 minutes.

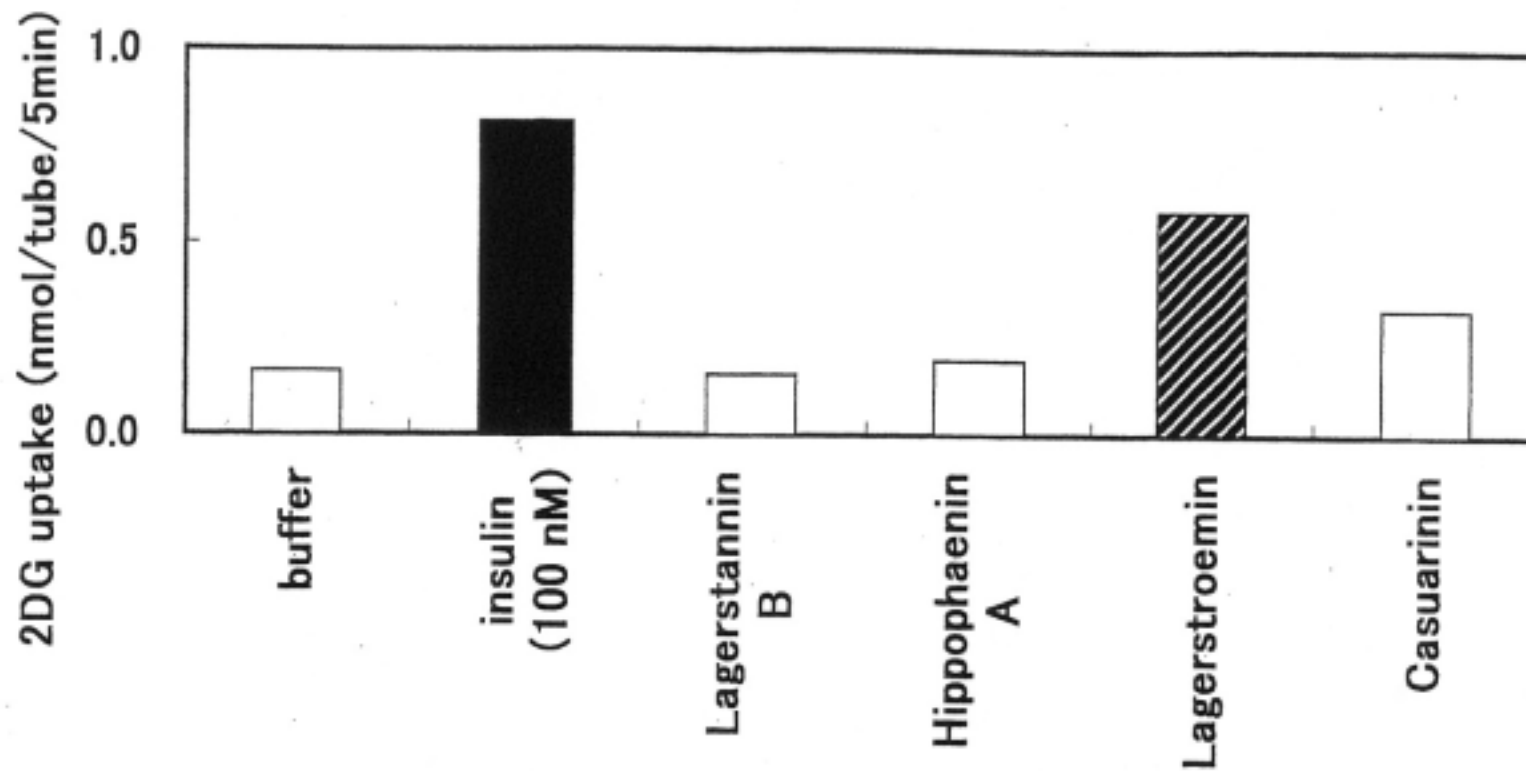


Figure 2. Glucose uptake enhancement activity of ellagitannins contained in banaba leaves

The free adipocytes were stimulated for 20 minutes with buffer, insulin (100nM) or other tannins (0.15mM) contained in banaba leaves shown above and the 2GD uptake amount was measured as shown in Figure 1.

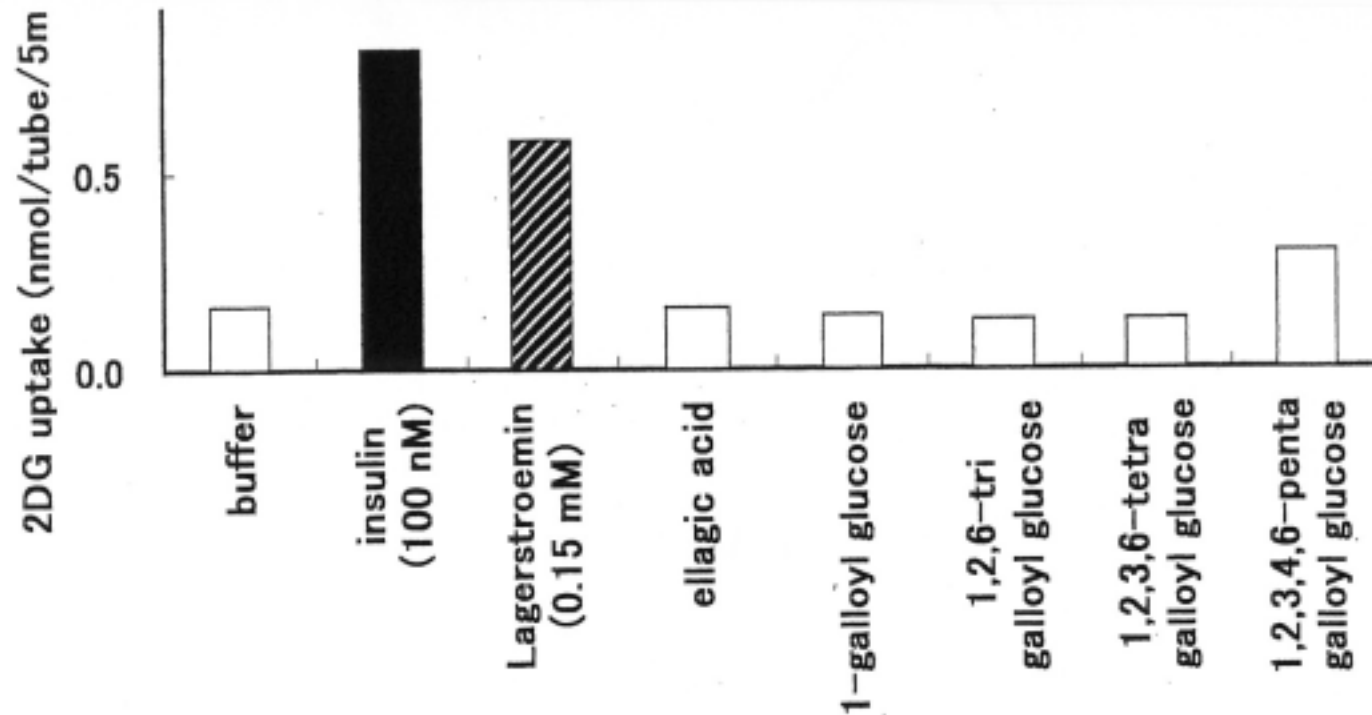
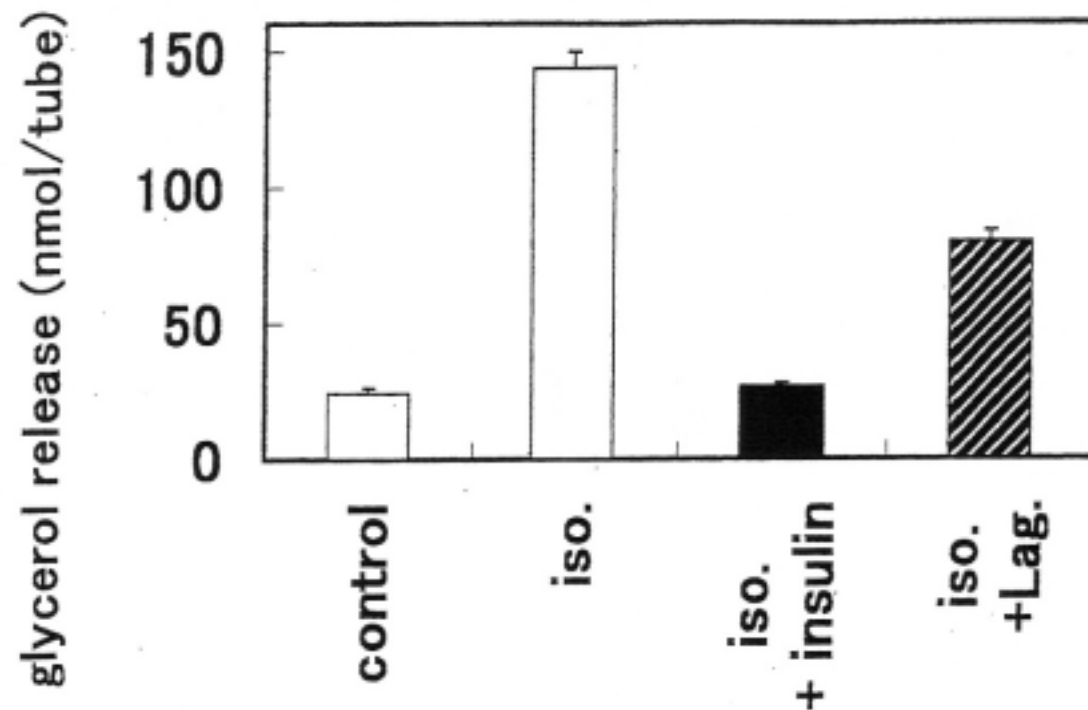


Figure 3. Glucose uptake enhancement activity of structure related compounds of Lagerstroemin

The free adipocytes were stimulated for 20 minutes with buffer, insulin (100nM), Lagerstroemin (0.15mM) or other structure related compounds of Lagerstroemin shown above, and the 2GD uptake amount was measured as shown in Figure 1.



First, we added buffer (control), 100nM insulin and 0.15mM Lagerstroemin (Lag.) to the free adipocytes. After stimulating the cells for 10 minutes, we added 100nM isoproterenol (iso.) to them and incubated them for 30 minutes. After stopping reaction with HClO_4 , we measured the free glycerol amount by fluorescence measurement.

Figure 4. Insulin-like activity of Lagerstroemin (2) (Lipid degradation control activity)

Lipid degradation control activity in adipose tissues is one of the physiological activities of insulin. Lagerstroemin also has the lipid degradation control activity besides the glucose uptake enhancement activity.

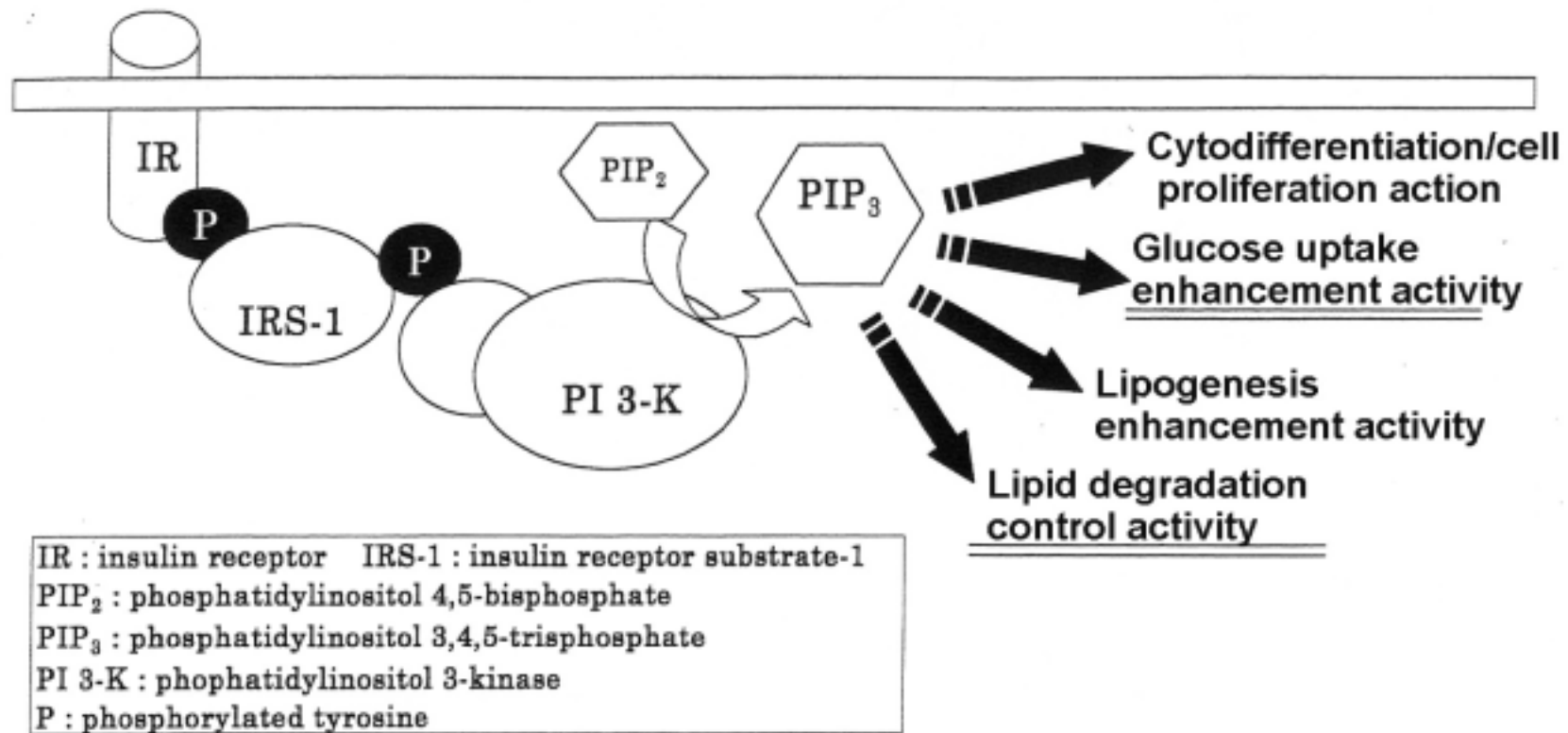


Figure 5. Signal transfer pathway of insulin in cells

The signal transfer pathway of insulin is believed to be like the figure illustrated above. Accordingly, we decided to pay attention to insulin receptor, insulin receptor substrate-1 and PI3-kinase when analyzing the mechanism of Lagerstroemin.

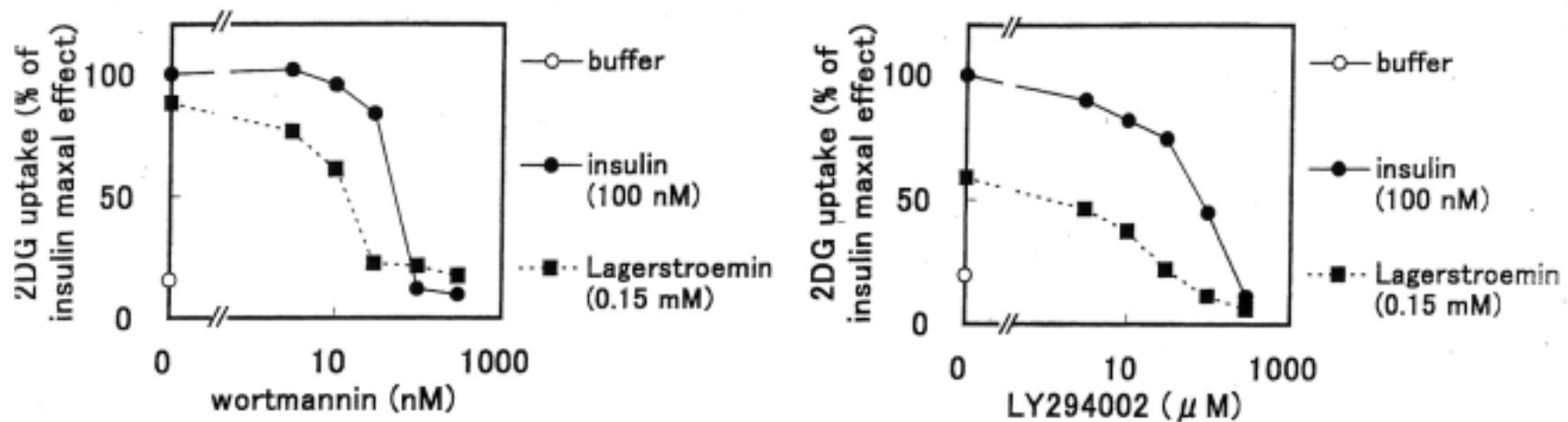
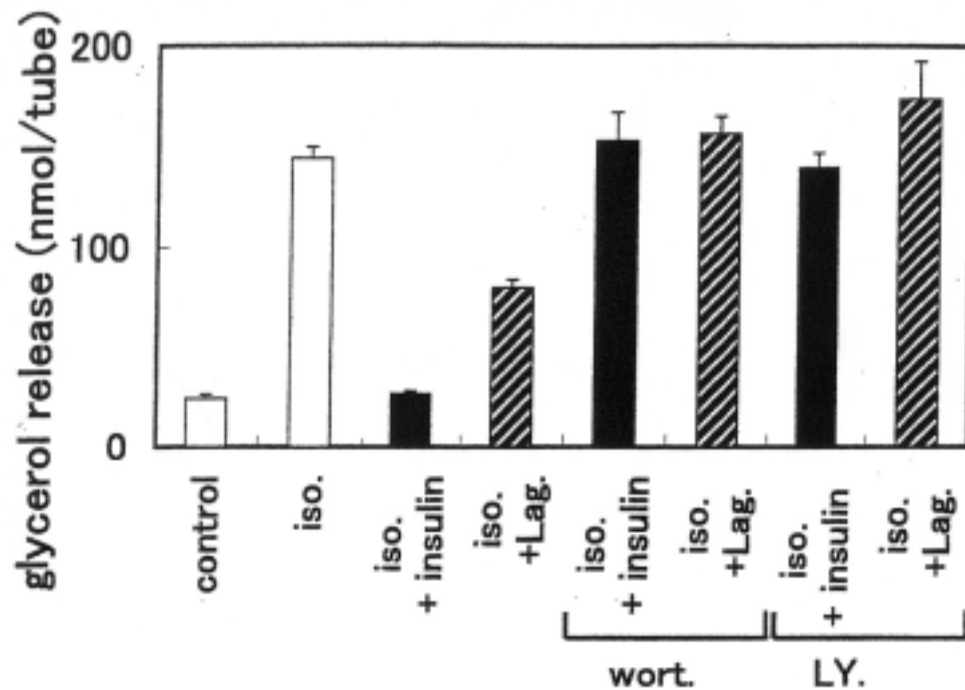


Figure 6. Influence of PI3-kinase inhibitor on glucose uptake enhancement activity of Lagerstroemin

First, the free adipocytes were pre-treated for 10 minutes with wortmannin and LY294002 of the concentration shown in the figure. Then, they were stimulated with insulin or Lagerstroemin for 10 minutes and the amount of 2DG uptake were measured as shown in Figure 1. Regarding 2DG uptake amount under each condition, we indicate the 2DG amount, which is taken in by insulin stimulation without inhibitor, as 100%.

Glucose uptake enhancement activity of Lagerstroemin was completely inhibited by wortmannin and LY294002 that are unique inhibitors of PI3-kinase as well as the activity of insulin is inhibited by them.



The free adipocytes were pre-treated with buffer, 100nM wortmannin (wort.) or 250 μ M LY294002 (LY.) for 10 minutes respectively. After adding 100nM insulin, 0.15mM Lagerstroemin (Lag.) to the cells and stimulating them for 10 minutes, we added 100nM isoproterenol (iso.) to the cells and incubated them for another 30 minutes. After using HClO₄ to stop the reaction, we measured the free glycerol amount by fluorescence measurement.

Figure 7. The influence of PI3-kinase inhibitor on the lipid degradation control activity of Lagerstroemin

The lipid degradation control activity of Lagerstroemin was also completely inhibited by wortmannin and LY294001 as well as the activity of insulin is inhibited by them.

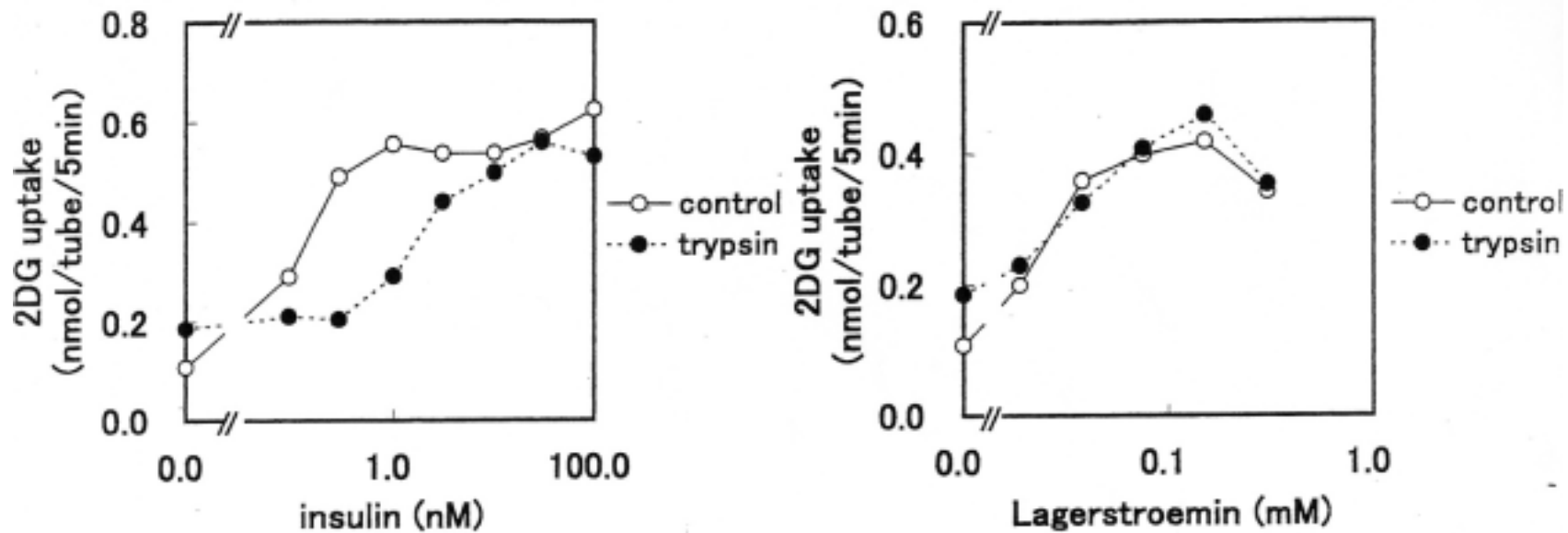


Figure 8. The influence of trypsin on glucose uptake enhancement activity of Lagerstroemin

The adipocytes were pre-treated with trypsin (8.3mg/ml) () or buffer () for 10 minutes. After stimulating them with insulin or Lagerstroemin of the concentration illustrated in the chart for 20 minutes, we measured the 2DG uptake amount as shown in Figure. 1.

By pre-treating the adipocytes with trypsin, you can partly break insulin receptors on the cell surface. Under this condition, the activity of Lagerstroemin was not influenced, although the glucose uptake enhancement activity of insulin depending on the concentration shifted to the higher concentration level.

Discussion

1. We found that tannins including Lagerstroemin showed insulin-like glucose uptake enhancement activity in adipocytes that are physical target tissues of insulin.
2. It appears that Lagerstroemin affects some part of signal transfer pathway of insulin because it showed anti lipid degradation control action besides glucose uptake enhancement activity.
3. It was inferred that Lagerstroemin worked the upper stream of PI3-kinase because the activity was completely inhibited by wortmannin and LY294002.
4. In the experiment using trypsin, it was suggested that the signal transfer pathway of Lagerstroemin was different from that of insulin. Until now, phosphorylation of insulin receptor or insulin receptor substrate has not seen yet. However, the reason can be that the action of Lagerstroemin is weaker than that of insulin and the substance doesn't reach to the detection limit.