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Effect of Alloxan on the Incorporation of Uridine Diphospho-D-Galactose into Cultured Pancreatic Endocrine Cells of the Rat

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The effect of alloxan on cultured rat pancreatic endocrine cells was investigated by following changes in the incorporation of UDP-galactose into glycoproteins(chloroform/methanol-insoluble fraction) and glycolipids(chloroform/methanol-soluble fraction).

Cultured cells exposed to either alloxan or alloxan plus D-glucose anomers (16.7 mM) at 37° for 5 min, were incubated in a medium containing labeled UDP-galactose for 60 min. Alloxan at concentrations of 1.25 or 6.25 mM had no marked effect on the incorporation of UDP-galactose but 31.2 mM alloxan markedly inhibited the incorporation. The combination of 1.25 mM alloxan and the α anomer of D-glucose significantly reduced the incorporation of UDP-galactose into glycoproteins and glycolipids, while the presence of the β anomer with alloxan had no effect.

Incorporation of D-[U-^{14}C]galactose was unaffected by 1.25 mM alloxan or alloxan plus D-glucose anomers (16.7 mM).

The present data indicate that cultured rat pancreatic endocrine cells can transfer galactose from UDP-galactose and that the α anomer reinforces the action of alloxan to a greater extent than the β anomer.

Keywords——cell surface galactosyltransferase; cultured pancreatic endocrine cells; glycoprotein; glycolipid; alloxan; α-D-glucose; β-D-glucose

Introduction

Alloxan has been widely used to induce diabetes in animals, but the exact mechanism by which alloxan destroys pancreatic B cells remains to be elucidated. Recently, the activity of galactosyltransferase on the outer surface of rat islets of Langerhans was found to be severely inhibited by alloxan, and this effect of alloxan was abolished to a considerable extent in the presence of the α anomer of D-glucose but not in the presence of the β anomer.[2)] These results seem to support the view that the α anomer and alloxan may compete for a common site located on the plasma membrane, *i. e.*, the glucoreceptor.[3,4)]

In the present work, we studied the characteristics of cell surface galactosyltransferase by following the changes in the incorporation of uridine diphospho-D-[U-^{14}C]galactose into cultured rat pancreatic endocrine cells exposed to either alloxan or alloxan plus D-glucose anomers.

Materials and Methods

Reagents——All reagents used were of special grade. Collagenase (type 1), α-D-glucose, β-D-glucose and tris(hydroxymethyl)aminomethane were purchased from Sigma Chemical Co., USA. Trypsin (1:250) was from Difco, USA. Bovine serum albumin (fraction V) was from the Armour Laboratories, USA. Medium

1) Location: *1-1, Mukogawa-cho, Nishinomiya, 663, Japan.*
2) Y. Tsumura, K. Murakoso, S. Kagawa, and A. Matsuoka, *Horm. Metab. Res.*, in press.
3) T. Tomita, P.E. Lacy, F.M. Matschinsky, and M.L. McDaniel, *Diabetes*, **23**, 517 (1974).
4) A.S. Pagliala, S.N. Stillings, W.S. Zawalich, A.D. Williams, and F.M. Matschinsky, *Diabetes*, **26**, 973 (1977).

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199 with Hanks' salt was from Grand Island Biological Co., USA. Fetal bovine serum was from Flow Laboratories, Australia. Penicillin G potassium salt (10^5 U/vial) and streptomycin sulfate (titer, 1 g/vial) were from Meiji Seika Co., Japan. UDP-[U-^{14}C]galactose (347 mCi/mmol) and D-[U-^{14}C]galactose (95 mCi/mmol) were from the Radiochemical Centre Amersham, England. PCS solubilizer was from Amersham Co., USA. D-Glucose, $MnCl_2$, alloxan and other reagents were products of Wako Pure Chemical Industries, Ltd., Japan.

Monolayer Cell Culture of Newborn Rat Pancreas——Monolayer cell culture of pancreas from newborn rats was performed according to a modification of the method of Lambert *et al.*[5] as described in our previous report.[6] Briefly, the pancreases of newborn Sprague-Dawley rats aged 2 to 4 days were dissected and minced, then the pancreatic tissues were treated in a mixture of trypsin (0.2%) and collagenase (0.3%) in Ca^{2+}- and Mg^{2+}-free phosphate buffer (7.7 mM, pH 7.4) containing 137 mM NaCl, 2.7 mM KCl and 2.8 mM glucose at 37°. This trypsin-collagenase treatment was repeated five times and each of the supernatants was transferred to a culture medium containing 10% fetal bovine serum, 91 mU/ml of penicillin and 10 μg/ml of streptomycin. Isolated cells were collected and resuspended in culture dishes (Falcon plastic, code 3003, Becton Division, Dickinson and Company, USA). The primary cell culture was performed at 37° under an atmosphere of 95% air and 5% CO_2. After culture for 20 hr, each cell suspension was transferred to new culture dishes. The final concentration of glucose in the culture medium was 16.7 mM.

Preparation of Cultured Pancreatic Cells——The cells, after culture for 2—3 days, were dissociated in phosphate-buffered saline (7.7 mM, pH 7.4, Ca^{2+}, Mg^{2+}-free) containing 10 mM EDTA as described in our previous report.[6] The suspended cells were washed with Krebs-Henseleit buffer (pH 7.4, KHB buffer), and resuspended in an appropriate volume of KHB buffer. Most of the cultured cells consisted of pancreatic endocrine cells.[6]

Incorporation of Galactose into Glycoproteins and Glycolipids——The medium used was buffer A, which consisted of 5.0 mM Tris, 139 mM Na^+, 4.7 mM K^+, 2.6 mM Ca^{2+}, 1.2 mM Mg^{2+} and 153.3 mM Cl^-. The suspended cells were placed in microplastic tubes and collected by centrifugation at $150 \times \boldsymbol{g}$ for 2 min. Each batch of cells (approximately 10^5) was equilibrated in 500 μl of KHB buffer containing 2.8 mM glucose and 0.5% bovine serum albumin at 37° for 30 min. The cells were treated with alloxan alone or alloxan plus D-glucose anomers in 500 μl (final volume) at 37° for 5 min and then washed twice with 2 ml of KHB buffer. Each batch of pretreated cells was incubated in 200 μl of buffer A supplemented with 1 mM $MnCl_2$ and UDP-[U-^{14}C]galactose (3.2 μM, 4.5×10^5 dpm) at 37° for 60 min. Labeled cells were disrupted by sonication for 30 sec at setting 3 on a Sonifier (80 W) (Branson Industries, USA) in 250 μl of the medium. One hundred μl of each sonicated sample was transferred to 500 μl of 10% TCA and centrifuged at $10^4 \times \boldsymbol{g}$ for 5 min. After adding 50 μl of 1 N NaOH and subsequently 1 ml of 5% TCA to the pellet, the suspension was centrifuged at $10^4 \times \boldsymbol{g}$ for 5 min. Precipitated materials were extracted twice with 500 μl of chloroform/methanol (2: 1, v/v); the extracts soluble in chloroform/methanol contained glycolipids and the residue contained glycoproteins. Chloroform and methanol were removed from the glycolipids by heating at 100° for 5 min in a scintillation vial. Glycoproteins and glycolipids were each dissolved in PCS–xylene (2: 1) and their radioactivities were determined in a liquid scintillation counter (Packard 3385, Packard Instrument Company Inc., USA). The blank value of the reaction, which was obtained by incubating cells boiled at 100° for 3 min prior to incubation with UDP-[U-^{14}C]galactose, was subtracted from each measurement. Experiments with D-[U-^{14}C]-galactose (51.4 μM, 2×10^6 dpm) were carried out by the same procedures.

Determination of the Number of Cultured Cells——The number of cultured cells was determined by light microscopy.

Calculation——Student "*t*" test was used to determine the statistical significance of differences.

Results

Effects of pH on the Incorporation of D-[U-^{14}C]Galactose and UDP-[U-^{14}C]Galactose

The pH was varied within the range of 6.0 to 7.4 in increments of 0.2 pH units. The incorporation of D-[U-^{14}C]galactose did not change with change of pH, while the incorporation of UDP-[U-^{14}C]galactose into glycoproteins and glycolipids reached a maximum at pH 6.8 (Fig. 1).

Effects of Various Concentrations of Alloxan on the Incorporations of D-[U-^{14}C]Galactose and UDP-[U-^{14}C]Galactose

The incorporation of D-[U-^{14}C]galactose into glycoproteins and glycolipids was unaffected by 1.25 mM alloxan, but was significantly reduced by either 6.25 or 31.2 mM alloxan. Alloxan

5) A.E. Lambert, B. Blondel, Y. Kanazawa, L. Orci, and A.E. Renold, *Endocrinology*, **90**, 239 (1972).
6) K. Murakoso, Y. Tsumura, S. Kagawa, and A. Matsuoka, *Chem. Pharm. Bull.*, **28**, 837 (1980).

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of 1.25 or 6.25 mM concentration had no effect on galactose transfer from UDP-[U-^{14}C]galactose, while at 31.2 mM it markedly inhibited the incorporation (Fig. 2).

Effect of Incubation Time on Galactose Transfer from UDP-[U-^{14}C]Galactose to Intact or Alloxan-treatcd Cells

Galactose transfer from UDP-[U-^{14}C]galactose to intact or alloxan-treated cells (exposed

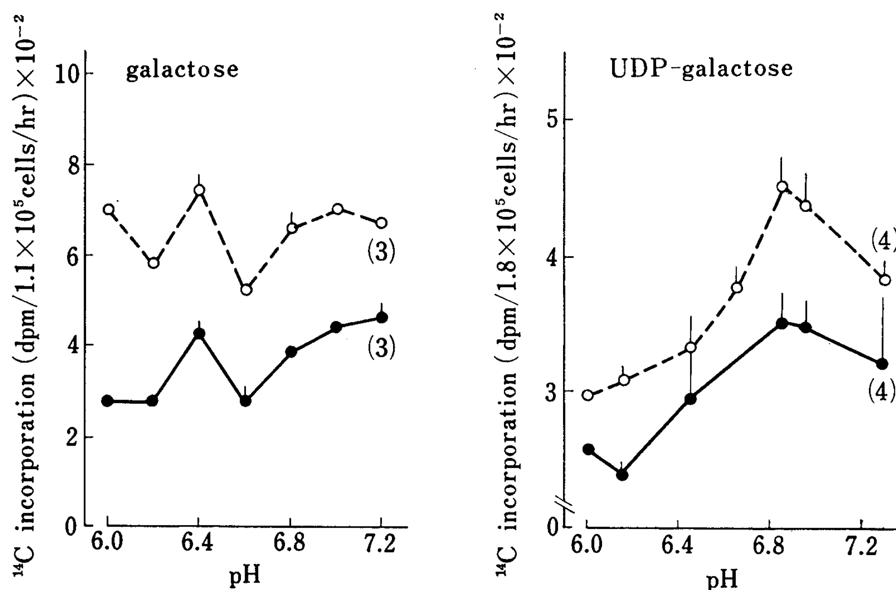

Fig. 1. Effect of pH on the Incorporations of Labeled Galactose from D-[U-^{14}C]Galactose and UDP-[U-^{14}C]Galactose

Cultured pancreatic endocrine cells (approximately 10^5) were incubated in buffer A containing 1 mM Mn^{2+} with D-[U-^{14}C]galactose (left) or UDP-[U-^{14}C]galactose (right) for 60 min at 37°. Incorporations into glycoproteins and glycolipids were determined as described in "Materials and Methods." Closed circles represent glycoproteins, and open circles glycolipids. Each point represents the mean (±SEM) of four observations.

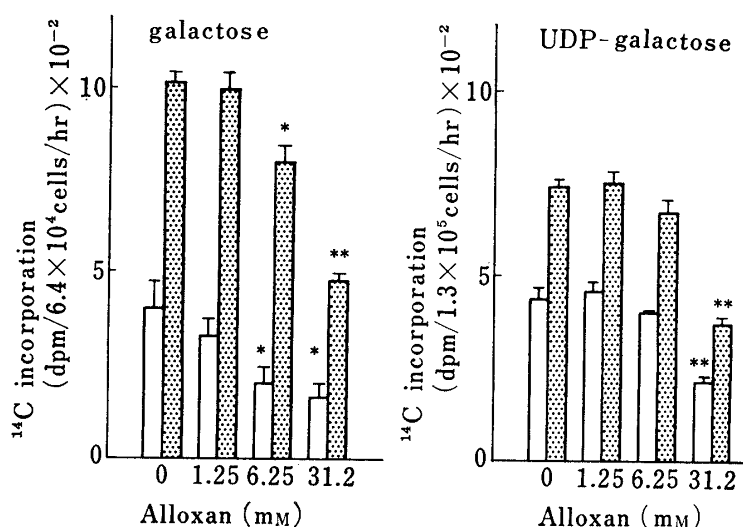

Fig. 2. Effect of Alloxan on the Incorporations of D-[U-^{14}C]Galactose and UDP-[U-^{14}C]Galactose

Cultured pancreatic endocrine cells were pretreated with various concentrations of alloxan for 5 min and incubated with D-[U-^{14}C]galactose (left) or UDP-[U-^{14}C]galactose (right) for 60 min. Open columns represent glycoproteins, and hatched columns glycolipids. Each value represents the mean (±SEM) of four observations. Statistical comparison was made against suitable controls. *$p < 0.05$, **$p < 0.001$.

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to 1.25, 6.25 or 31.2 mM alloxan for 5 min prior to incubation) is shown as a function of incubation time in Fig. 3. Incorporation of galactose into glycoproteins reached a plateau at 60 min, while that into glycolipids, except in the case of 31.2 mM alloxan, was a nearly linear function of incubation time. After incubation for 180 min, the ratio of incorporation into glycoproteins to that into glycolipids was around 4.5 in intact and alloxan-treated cells except for the cells treated with 31.2 mM alloxan, in which the ratio was 9, indicating that a high concentration of alloxan inhibited the incorporation into glycoproteins more significantly than that into glycolipids ($p<0.01$).

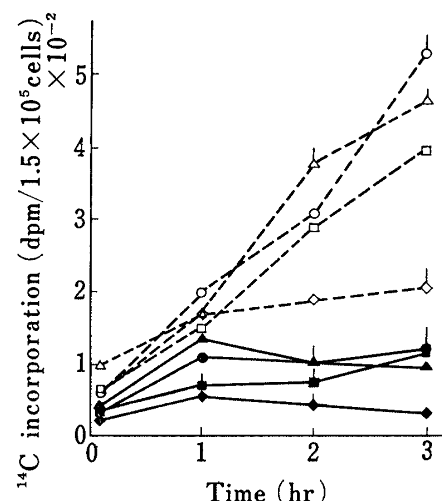

Fig. 3. Effect of Incubation Time on Galactose Transfer from UDP-[U-^{14}C]Galactose to Intact or Alloxan-treated Cells

Cultured pancreatic endocrine cells (approximately 10^5) were pretreated with various concentrations of alloxan (mM) (0; ●○, 1.25; ▲△, 6.25; ■□, 31.2; ◆◇) for 5 min at 37° and then incubated with UDP-[U-^{14}C]galactose for 3 hr. Incorporations into glycoproteins and glycolipids were determined as described in "Materials and Methods." Solid lines represent glycoproteins, and dotted lines glycolipids. Each point represents the mean (±SEM) of four observations.

Effects of Alloxan Plus D-Glucose Anomers on the Incorporations of UDP-[U-^{14}C]Galactose and D-[U-^{14}C]-Galactose

Cultured pancreatic cells were treated with either 1.25 mM alloxan or alloxan plus D-glucose anomers at 16.7 mM concentration for 5 min. Incorporation of D-galactose was unaffected by either alloxan or alloxan plus the anomers. With UDP-galactose, the α anomer in the presence of alloxan significantly reduced the incorporation of galactose into both glycoproteins and glycolipids, while the β anomer had no effect (Fig. 4). The combination of 31.2 mM alloxan and 16.7 mM glucose anomers inhibited the incorporation of galactose to the same extent as did alloxan alone, so neither anomer produced a synergistic

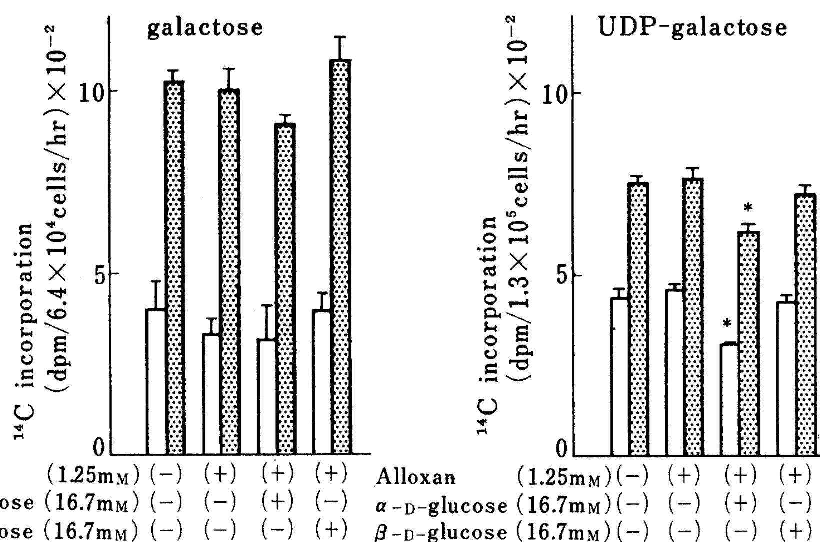

Fig. 4. Effects of Alloxan plus D-Glucose Anomers on the Incorporations of D-[U-^{14}C]Galactose and UDP-[U-^{14}C]Galactose

Cultured pancreatic endocrine cells were pretreated with 1.25 mM alloxan or alloxan plus D-glucose anomers of 16.7 mM concentration for 5 min and then incubated with D-[U-^{14}C]galactose (left) or UDP-[U-^{14}C]galactose (right) for 60 min. Open columns represent glycoproteins, and hatched columns glycolipids. Each value represents the mean (±SEM) of four observations. Statistical comparison was made against groups treated with 1.25 mM alloxan. *$p<0.05$.

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effect on the incorporation of UDP-[U-^{14}C]galactose (Fig. 5).

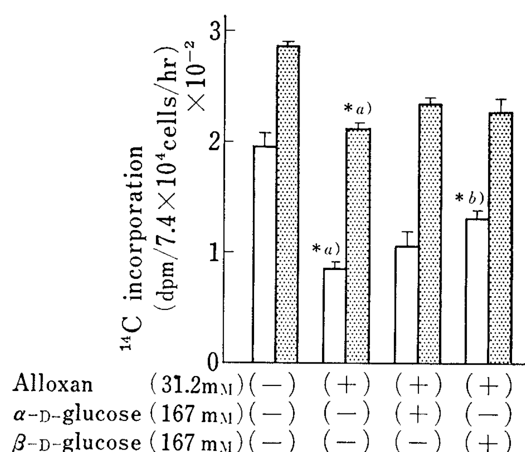

Fig. 5. Effect of Alloxan plus D-Glucose Anomers on the Incorporation of UDP-[U-^{14}C]Galactose

Cultured pancreatic endocrine cells were pretreated with 31.2 mM alloxan alone or alloxan plus D-glucose anomers at 16.7 mM concentration for 5 min and then incubated with UDP-[U-^{14}C]galactose for 60 min. Open columns represent glycoproteins, and hatched columns glycolipids. Each value represents the mean (±SEM) of four observations. Statistical comparison was made against the control (*a*) and the alloxan-treated group (*b*). *$p < 0.001$.

Discussion

Roseman demonstrated that glycosyltransferases can catalyzed the transfer of sugars from nucleotide sugars to acceptors located on the cell surface and that the enzymes are also a part of cell surface receptors functioning in cellular adhesion.[7] Since nucleotide sugars are substrates specific for cell surface glycosyltransferases, a study of the changes in the incorporation of UDP-[U-^{14}C]galactose caused by alloxan should cast light on the mechanism of alloxan action on the cell membranes.

Incorporation of UDP-galactose was dependent on pH of the medium, in contrast to that of D-galactose. It is well known that the activity of outer surface galactosyltransferases is pH dependent and also is affected by divalent cations such as Ca^{2+} and Mg^{2+}.[6] Incorporation of galactose into glycoproteins and glycolipids was 1.4 to 2.4 times greater with UDP-galactose than with D-galactose. This may reflect the higher activity of galactosyltransferases in the outer surface compared to that in the Golgi apparatus, since nucleotide sugars cannot pass the cell surface.[8] There is also a possibility that the conversion of D-galactose to UDP-galactose may be relatively slow in cultured pancreatic endocrine cells. It is clear that alloxan at relatively high concentrations inhibited galactosyltransferase activity both on the cell surface and in intracellular organelles, in view of the reduction in the incorporations of D-galactose and UDP-galactose by alloxan. The concentrations of alloxan causing inhibition of galactosyltransferase activity, 6.25 and 31.2 mM, seem to be relatively high as compared to the concentration at which alloxan inhibits insulin release and biosynthesis. Recently, Orci *et al.*[9] demonstrated that alloxan at a relatively high concentration, 6.25 mM, produced a significant decrease in the number of membrane-associated particles in rat islets of Langerhans, and that D-glucose prevented the ultrastructural alteration of the plasma membrane. Therefore, it seems likely that the reduction in the activity of outer surface galactosyltransferase by alloxan is associated with changes of morphological organization, including the fluidity of the membrane structure. Moreover, the α anomer of D-glucose synergistically enhanced the inhibitory effect of alloxan on the incorporation of UDP-galactose in contrast to the β anomer, while alloxan did not show enhaced inhibition of the incorporation of D-[U-^{14}C]galactose in the presence of the anomers. These observations seem to support the hypothesis that the outer surface of cultured endocrine cells may distinguish the anomers of D-glucose and that the binding sites for alloxan may be closely related to those for the α anomer of D-glucose. However, this effect of the α anomer is in contrast to the specifically protective effects of the α anomer mentioned earlier. Weaver *et al.*[10] reported recently that D-glucose enhances the

7) S. Roseman, *Chem. Phys. Lipids*, **5**, 270 (1971).
8) L.M. Patt and W.J. Grimes, *J. Biol. Chem.*, **249**, 4157 (1974).
9) L. Orci, M. Amherdt, F. Malaisse-Lagae, M. Ravazzola, W.J. Malaisse, A. Perrelet, and A.E. Renold, *Lab. Invest.*, **34**, 451 (1976).
10) D.C. Weaver, M.L. McDaniel, and P.E. Lacy, *Endocrinology*, **102**, 1847 (1978).

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uptake of labeled alloxan into the islets. Therefore, the above finding may reflect nonspecific actions of alloxan; the presence of the α anomer may lead to a greater alteration in the conformation of the cell membrane (followed by changes in the fluidity of the cell membranes) than the presence of the β anomer, giving alloxan molecules relatively free access to the cell membrane. In addition, there is also the possibility that alloxan may react not only with outer surface galactosyltransferase, which is a glycoprotein, but also with glycoproteins and glycolipids as acceptor molecules for galactosylation in view of the finding that alloxan impairs the Na,K-ATPase system in rat islets of Langerhans,[11] which has been demonstrated to be a glycoprotein.[12]

In conclusion, the present data indicate that cultured pancreatic endocrine cells possess cell surface galactosyltransferase and that the α anomer of D-glucose synergistically enhances the inhibitory effect of alloxan on the incorporation of UDP-galactose into glycoproteins and glycolipids.

11) J.C. Henquin, P. Malvauux, and A.E. Lanbert, *Diabetologia*, **16**, 253 (1979).
12) L.E. Hokin, *Ann. N.Y. Acad. Sci.*, **242**, 12 (1974).

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