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Response of dairy cows to glucose administration

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乳牛에 있어서 葡萄糖 投與의 反應에 關한 實驗的 硏究

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초 록 : 實驗對象으로서 乳牛 5頭에게 50% 葡萄糖 500㎖를 生理的食鹽水에 混合 13~15回 反復 경정맥(jugular vein)을 통해 注入後 血中 insulin水準과 Langerhans islets의 組織化學을 觀察한바, 血中 *insulin*의 水準은 葡萄糖의 投與前에 比하여 4~11倍나 增加됨이 觀察되었다.

한편, Langerhans islet의 B cell도 葡萄糖의 投與回數에 따라 特異하게 주름이 觀察되었는데 이는 B cell에서의 insulin 合成能을 높이는데 그 原因이 있었다고 본다.

Key words : cow, glucose, insulin, B cell.

Introduction

Circulating insulin, which is of importance in carbohydrate metabolism in dairy cows, is known to trigger ketosis. During postpartum astasia in dairy cows the blood insulin level is reduced[4,8] and carbohydrate preparations used to be administered for differential diagnosis in ketosis and postpartum astasis.[2,10] After glucose administration intermediate metabolites are produced by phosphorylation or nicotinamide-adenine-dinucleotide phosphate derivatives.[10]

In this study, the blood insulin respons in dairy cows after glucose administration was evaluated in relation with histochemistry of Langerhans islets in the pancreas.

Materials and Methods

Six Holstein cows weighing 560 to 710 kg in the late lactating period were used. They were kept at three farms in an eastern district of Saitama Prefecture and experienced two to four deliveries.

Five hundred milliliters of 50% glucose in physiologic saline(Nihon Zenyaku Kogyo, Kooriyama) was administered via the jugular vein over 13 to 15 min. Blood was collected from the immediately before and after administration and examined for enzyme activities using a Glzyme (Eiken Kagaku, Tokyo), and for insulin by a double antibody method[6,7] using a ^{125}I kit(Dynabot, Tokyo).

The cows were exsanguinated at a slaughter house within immediately after glucose administration, Immediately, the pancreas was sampled, excised and fixed in neutral formalin. The tissues were then embedded in

paraffin, and sections were made and stained with hematoxylin-eosin, Gomori's aldehyde-fuchsin, Grimelium method, or Hellman-Hellerstrom method. Same sections were subjected to immunohistochemistry for insulin and glucagon by avidin-biotin complex method[5] using guinea pig antibodies and peroxidase-labeled anti-IgG (ICN Biomedicals, USA). For electron microscopy pancreatic tissues were fixed in Karnovsky's solution, embedded in Epon 812. Ultra-thin sections were made and stained with uranyl acetate[12] and lead nitrate.[9] They were observed using a JEM-100CX (Nihon Denshi, Tokyo).

Results

Immediately before glucose administration the mean blood insulin level was $20.0 \pm 7.7\ \mu U/m\ell$, which was increased markedly to $115.3 \pm 27.3\ \mu U/m\ell$ ($p < 0.05$) after administration. Case A showed a high insulin level before glucose administration, which markedly increased after administration. In other five cases, blood insulin level remarkably increased immediately after glucose administration.

In case B and D, it attained about 11 times the pre-administration level.

The blood glucose levels after administration ranged from 249 to 361 mg/dℓ, being 4 to 6 times higher as compared with those before administration(Table 1).

The Langerhans islets of each cow were positive for aldehydefuchsin and intensely with combined anti-insulin antibody, while they weakly reacted with anti-glucagon antibody. The islets of case A, showing high blood inuslin level as well as cases B and D showing normal level before glucose administration(Figs 1 to 6), equqlly staines with aldehyde-fuchsin and reacted with anti-insulin antibody after glucose administration.

Electron microscopy revealed that endocrine B cells frequently containied needle-shaped granules in the electron-dense cytoplasm whereas A cells containing relatively smaller granules in the less electron-dense cytoplasm. Both type of cells showed no abnormalities(Fig 7).

The cytoplasm of exocrine gland cells, had dilated rough endoplasmic reticulum various zymogen granules on the luminal side. Mitochondria tended to be swollen and secondary lysosomes and fat droplets were infrequently noted. No abnormalities were noted in centroacinar cells or interlobular ductal cells.

Discussion

In lactating cows, the blood insulin showed the lowest level in the maximum lactation period thereafter increasing with decreasing lactation.[11] It also increases immediately before the onset of ketosis while decreases later. Hypoglycemia during lactation is observed with induced hyperinsulinemia, and ketosis can be induced under hypoinsulinemia.[8] Ketotic cows responded to glucose administration, showing a low blood insulin level, suggesting that ketosis might be associated with the dysfunction of pancreatic B cell.[4]

Boer et al[1] reported that the insulin concentration and the insulin/glucagon ratio decreased in ketosis. Herbein et al.[3] also reported that the blood suger, insulin, and insulin/glucagon ratio increased at the advanced lactation period although the insulin/glucagon ratio was redused matching the glucose demand at the early lactation period. Thus, the blood insulin level is closely related to ketosis in dairy cows.

Table 1. Responses of the blood insulin level and histochemistry of Langerhans islets after glucose administration

Case	Blood insulin($\mu U/m\ell$)		Histochemistry and immunohistochemistry of Langerhans islets					
	Befire glucose	After glucose	Hematoxylin -eosin	Aldehyde -fuchsin	Insulin	Glucagon	Grimelius	Hellerstrom -hellan
A	57	230	−*	++	+++	−/±	±	−
B	6	63	−	++	++	+	+	−
C	21	160	−	+	+	±/+	+	−
D	9	97	−	++	++	−/±	±/+	−
E	12	81	−	+	+	−/±	±	−
F	15	61	−	+	+	±	±/+	−

* − : Negative in all cells, ± : Clightly positive less than half of the cells, + : Modrately positive in about half of the cells, ++ : Modreately positive in more than half the cells, +++ : Highly positive in nearly all cells.

Insulin activates glucokinase of the liver and stimulates the glycolytic system by increased utilization of glucose. In this process, pyruvic acid was increased and converted to acetyl-CoA and then to oxaloacetic acid stimulating the TCA cycle, resulting in enhanced utilization of acetyl-CoA, increased production of fatty acids and reduced accumulation of ketone. Insulin also increases the uptake and utilization of glucose in various tissues, especially stimulating the synthesis of neutral fat in the adipose tissue. Such effects of insulin in the liver and adipose tissue lead to a reduction in free fatty acids in the blood and to nomalization of the blood suger level.

Legends for figures

Fig 1. Normal islets of case A. H-E. × 200.
Fig 2. Aldehyde-fuchsin stained(++) islets of cow A. × 200.
Fig 3. Islets reacting with anti-insulin antibody(+++) of cow A. × 200.
Fig 4. Islets reacting with anti-glucagon antibody (−/±) of cow A. × 200.
Fig 5. Islets reacting with anti-insulin antibody (++) of cow B. × 200.
Fig 6. Islets reacting with anti-glucagon antibody (+) of cow B. × 200.
Fig 7. Islet of Langerhans of cow B, showing B cells containing a number of needle-shaped granules in the electron-dense cytoplasm and A cells containing smaller granules in the less electron-dense cytoplasm. Electron microscopy. × 1,600.

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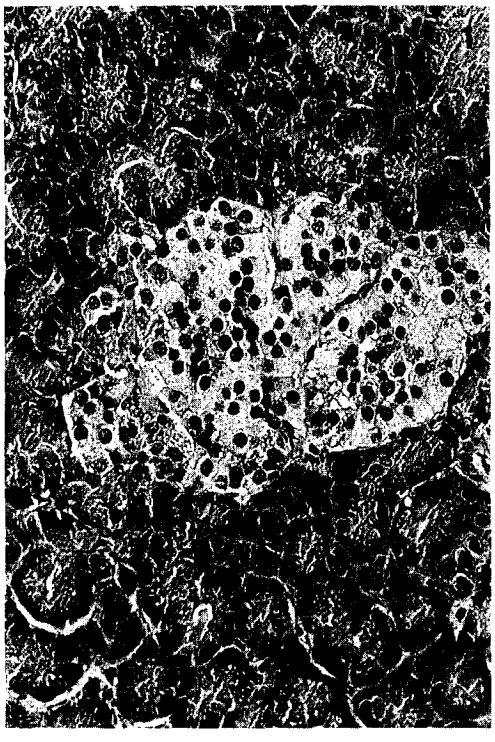

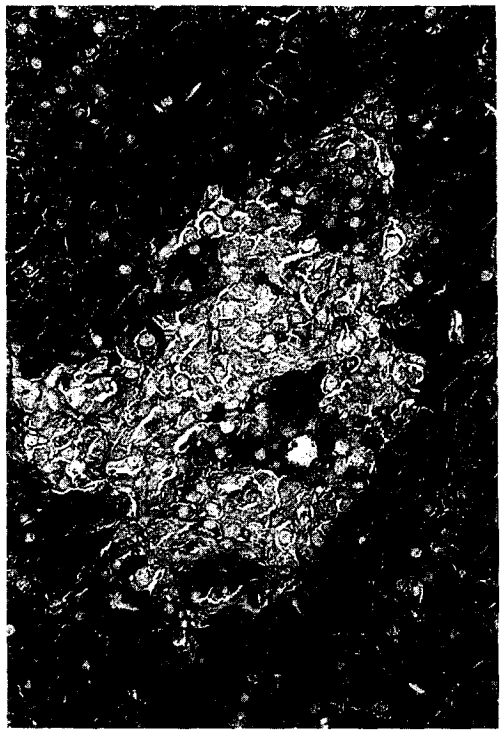

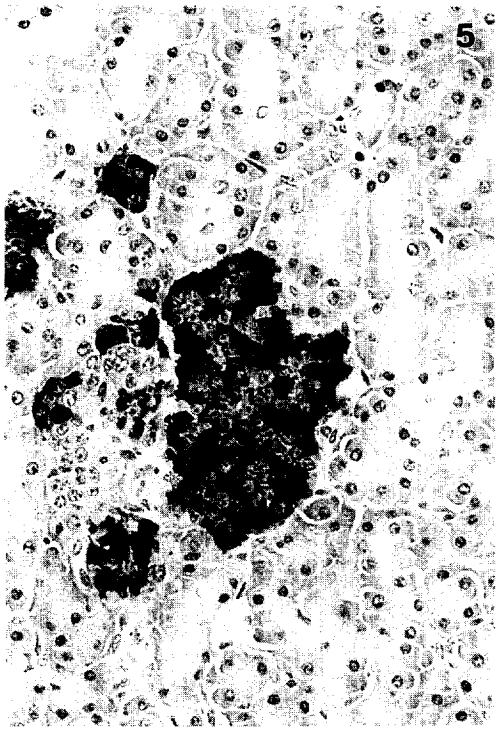
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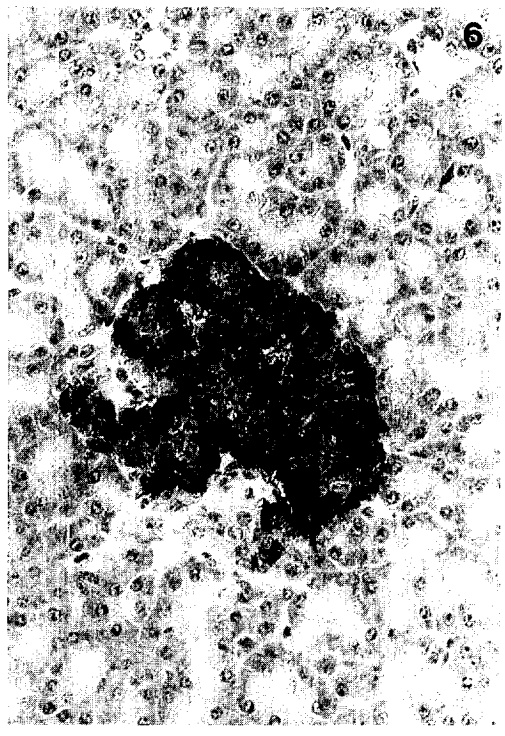
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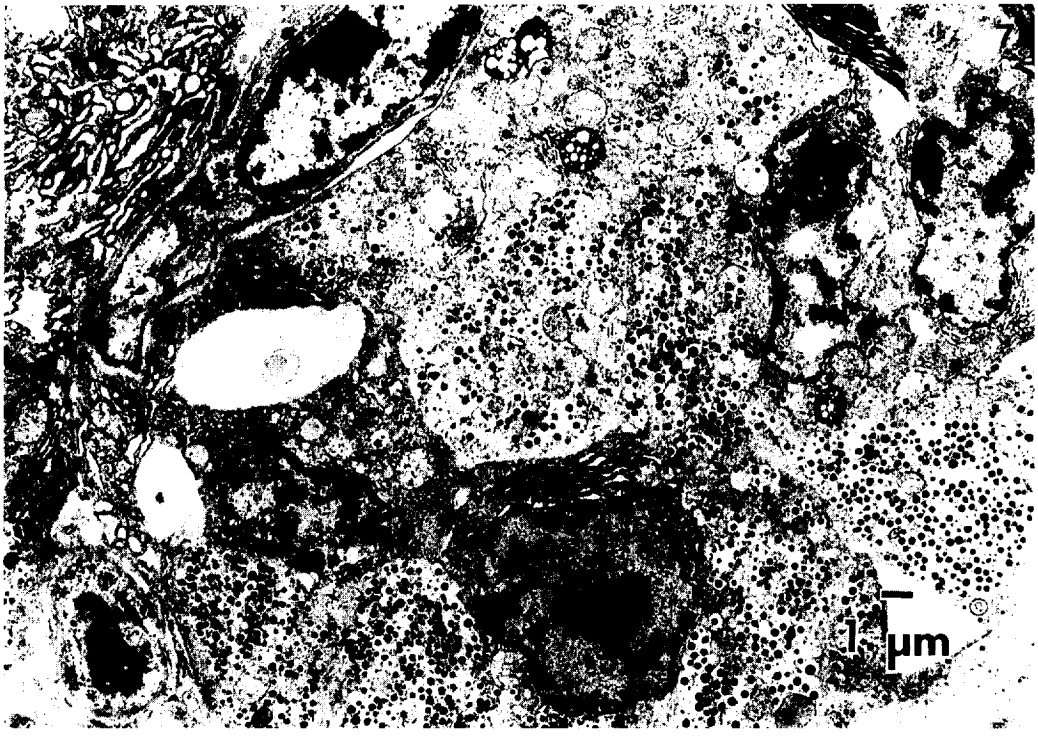
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