Propionate Tolerance Test for Determination of Insulin Secretion in a Hyperglycemic Japanese Black Steer

Masaki TAKASU1), Yasunori OHBA1)*, Tomonori IGUCHI1), Naohito NISHII2), Sadatoshi MAEDA1) and Hitoshi KITAGAWA1)

1)Department of Veterinary Medicine, Faculty of Applied Biological Sciences and 2)Department of Clinical Veterinary Medicine, United Graduate School of Veterinary Sciences, Gifu University, 1-1 Yanagido, Gifu 501-1193, Japan

(Received 7 February 2007/Accepted 30 May 2007)

ABSTRACT. A propionate tolerance test (PTT) was used to determine the pathophysiology of a Japanese Black steer with hyperglycemia. In the hyperglycemic steer, a low insulin secretion was confirmed by a glucose tolerance test (GTT), so that the hyperglycemic steer was diagnosed as insulin-dependent diabetes mellitus. Although the plasma insulin concentration in the control cattle increased in response to propionate stimulation, a low insulin response to PTT was observed in the diabetic steer. The fact that both PTT and GTT determined that the diabetic steer had low insulin secretion suggests that the PTT might be an effective diagnostic tool for diabetes mellitus in cattle.

NOTE: Internal Medicine

Diabetes mellitus is uncommon in cattle whose blood glucose concentration is regulated within a narrow range. Although there are a few individual reports of diabetes mellitus in cattle [1–4, 7–9], most of them deal mainly with histopathological findings of diabetic cattle [11–14]. Therefore, only a few clinical features of diabetes mellitus in cattle have been clarified. To determine the pathophysiology of diabetes mellitus, the intravenous glucose tolerance test (GTT) is commonly carried out in cattle as well as other animals, and diabetes mellitus is classified as either insulin-dependent or non-insulin-dependent [5, 6]. In ruminants, the blood glucose concentration is maintained at a stable level, because it is regulated by fermentation in the rumen and by gluconeogenesis from volatile fatty acids in the liver. Therefore, cattle are poorly adapted to hyperglycemia, and hyperglycemia caused by GTT might induce metabolic abnormality in diabetic cattle to make their condition worse. Since glucose metabolism in ruminants is quite different from that in monogastric animals, an alternative method of diagnosis of diabetes mellitus in cattle seemed to be needed. In this report, we conducted a propionate tolerance test (PTT) to determine the pathophysiology of a hyperglycemic steer, because propionate injection is known to directly stimulate insulin secretion and to be a major glycogenic substrate for ruminants [10].

A one-year-old Japanese Black steer, weighing 220 kg and in an emaciated condition, was presented with a 2-month history of chronic serous diarrhea, weight loss, dehydration, and depression. Its rectal temperature, heart rate, and respiratory rate were 39.4°C, 65 beats/min, and 18 breaths/min, respectively, and they were considered normal.

The steer’s water intake increased, and the frequency and strength of its rumen contractions decreased. The steer demonstrated neither the abnormal behavior associated with neurologic manifestations nor the posture peculiar to abdominal pain.

A routine hematological examination, including a complete blood count and blood biochemistry, was performed using an automatic analyzer (Celltac, MEK-5258, Nihon Kohden, Tokyo, Japan, and FDC 3500V, Fuji Medical Systems, Tokyo, Japan). The steer’s leukocyte count, erythrocyte count, and hematocrit value were 12.6 × 10³/µl, 10.2 × 10²/µl, and 47.0%, respectively. The serum glucose concentration was 286 mg/dl, while its serum total protein, albumin, and urea nitrogen concentrations were 9.2 g/dl, 3.8 g/dl, and 47.2 mg/dl, respectively. In addition, its serum aspartate aminotransferase and creatinine concentrations were 48.0 U/l and 1.2 mg/dl, respectively. The results of the complete blood count indicated that the steer had marginal leukocytosis, high erythrocyte count, and high hematocrit value. Moreover, the results of blood chemistry analysis revealed that the steer had hyperglycemia, hyperalbuminemia, high urea nitrogen concentration, and normal aspartate aminotransferase and creatinine concentrations. Therefore, hyperglycemia suggesting diabetes mellitus was a prominent feature in this steer. Moreover, dehydration was suspected from the results, which included high hematocrit value, hyperalbuminemia, and high urea nitrogen concentration in spite of anorexia. However, the liver and renal functions of the steer might be normal. Serum cortisol concentration (TFB Cortisol Kit, TFB, Tokyo, Japan) was determined to be less than 1.0 µg/dl, suggesting that the hyperglycemic steer might not be under stress that could lead to high glucose levels. In addition, glucosuria was confirmed by routine urinalysis. All data strongly suggested diabetes mellitus.
To determine the pathophysiology of the hyperglycemic steer, its plasma glucose and insulin concentrations were determined during a GTT [5]. A glucose solution (0.5 g/kg) was injected through an indwelling catheter into the jugular vein for 2 min. The 0 min of the tolerance test was established as the midpoint of the infusion. Blood samples were collected immediately before and at 5, 15, 25, 35, 45, 60, and 120 min after injection using a small volume of heparin sodium as an anticoagulant. The samples were centrifuged at 800 × g for 10 min immediately after blood sample collection and were stored at −80 °C until determination of glucose and insulin concentrations. The plasma glucose concentrations were determined using an automatic analyzer, and the plasma insulin concentrations were determined by ELISA (Mercodia Bovine Insulin ELISA, Mercodia, Uppsala, Sweden). Five Japanese Black cattle aged 13.0 ± 6.7 months old (2 castrated males and 3 females) from a commercial farm were used as a normal control. The changes in plasma glucose concentrations during the GTT are shown in Fig. 1. The plasma glucose concentration in the hyperglycemic steer rose as high as 738 mg/ml, while which in the control cattle remained at 487.0 ± 71.2 mg/ml. Moreover, the plasma glucose concentration in the hyperglycemic steer decreased quite slowly compared with the control cattle. The half-life of the glucose concentration in the hyperglycemic steer was 83.5 min, while it was 33.9 ± 9.4 min in the control cattle. The plasma insulin concentration in the control cattle increased rapidly in response to glucose injection. However, no such response was observed in the hyperglycemic steer, indicating that its insulin secretion was far lower than the control cattle. The hyperglycemic steer was diagnosed as insulin-dependent diabetes mellitus.

A PTT was carried out to determine the pathophysiology of the diabetic steer. A propionate solution (0.625 mmol/kg and pH 7.4) [10] was prepared and injected through an indwelling catheter into the jugular vein for 2 min. The 0 min of the tolerance test was established as the midpoint of the infusion. Blood samples were collected immediately before and at 5, 15, 25, 35, 45, 60, and 120 min after injection using a small volume of heparin sodium as an anticoagulant. The plasma glucose and insulin concentrations were determined in the same manner as for the GTT. Five Japanese Black cattle aged 7.3 ± 1.9 months old (3 castrated males and 2 females) from a commercial farm were used as a normal control. The changes in plasma glucose and insulin concentrations during the PTT are shown in Fig. 2. The plasma glucose concentration in the control cattle decreased after propionate injection, and returned to a normal level gradually. However, the plasma glucose concentration in the diabetic steer remained stable regardless of propionate stimulation. The plasma insulin concentration in the control cattle increased rapidly after propionate injection, whereas no such response was observed in the diabetic steer.

An insulin tolerance test (ITT) was carried out to determine the insulin sensitivity of the diabetic steer [5]. Regular insulin (Novolin R 100, Novo Nordisk, Denmark; 0.1 U/kg) was injected intramuscularly. Blood samples were collected immediately before and at 30, 60, 90, 120, 150, 180, 210, and 240 min after injection using a small volume of heparin sodium as an anticoagulant. The samples were centrifuged at 800 × g for 10 min immediately after blood sampling and were stored at 4 °C until the end of the ITT. The plasma glucose concentration was then determined using an automatic analyzer. Five Japanese Black cattle aged 9.3 ± 2.9 months old (3 castrated males and 2 females) from a commercial farm were used as a normal control. The changes in the plasma glucose concentrations are shown in Fig. 3. The exogenous insulin reacted effectively in the control cattle, and their lowest plasma glucose concentration was 43.2 ± 15.0 mg/dl at 120 min after injection. Although insulin administration took a much longer time to act in the diabetic steer, the plasma glucose concentration decreased gradually.

Diabetes mellitus in cattle is often immune-mediated, and there is frequently an underlying viral infection, usually bovine viral diarrhea virus (BVDV), that triggers an autoimmune reaction causing insulitis in pancreatic beta cells [1, 9,
Therefore, RT-PCR was carried out to detect BVDV in the leukocytes of the diabetic steer, and this eliminated BVDV infection as the cause of diabetes mellitus in the diabetic steer.

Injection of propionate into the control cattle stimulated insulin secretion, and this indicated that the PTT, in addition to the GTT, is capable of determining insulin secretion in cattle. Therefore, the PTT may have a role in evaluating insulin secretion in diabetic cattle.

Injection of a pharmacological dose of glucose (GTT) might have worsened the condition of diabetic ruminants including cattle, because ruminants adapt poorly to hyperglycemic condition. On the other hand, propionate is one of the glycogenic substrates for ruminants and is theoretically a much safer stimulator of pancreatic beta cells than glucose, and this is an advantage of the PTT in determining pathophysiology of diabetic cattle.

In this study, we used a PTT to evaluate insulin secretion in the diabetic steer, and the steer exhibited a poor insulin response to the propionate injection. Although further study is required, this case suggested that the PTT might be an effective diagnostic tool for diabetes mellitus in cattle.

REFERENCES