Effects of Lipoic Acid on Plasma Metabolites and Metabolic Response to Intravenous Injection of Isoproterenol in Broilers

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ABSTRACT : To examine the effects of lipoic acid on metabolic response to a β-agonist, isoproterenol, in broilers, chicks were fed dietary lipoic acid at levels of 0 (control) or 50 mg/kg for 24 d. At 27 d of age, chickens were randomly selected. Isoproterenol dissolved in 0.9% saline was injected into the wing vein at a dosage of 2 mg per kg BW; then, blood samples were taken at 0, 10, 20, 30, 60, 90, 120, and 180 min. Amounts of plasma glucose, NEFA, triglyceride and total cholesterol were determined. Dietary lipoic acid reduced only plasma total cholesterol by 25%. Following isoproterenol injection, plasma glucose in both groups increased for 20 min; then, it returned to its basal concentration. In contrast, the maximal reductions in plasma NEFA and triglyceride in both groups (20 to 30 min) were confirmed by isoproterenol injection. In addition, while glucose returned to the basal level, plasma NEFA in the lipoic acid-treated chickens increased above the basal or control value during the 60 to 180 min post-injection. The present study suggests that the dietary administration of lipoic acid elicits fatty acid mobilization in β-adrenergic response to isoproterenol when the basal level of plasma glucose is maintained. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 5 : 653-658*)

Key Words : Lipoic Acid, β-Agonist, Isoproterenol, Broiler Chicken, Plasma Free Fatty Acid

INTRODUCTION

A potent growth promoter, β-adrenergic agonist (β-agonist), is well known to increase protein accumulation and/or decrease adipose tissue accretion in domestic animals (Weppelman, 1984; Yang and McElligott, 1989) and illegal use of β-agonist in the meat-producing industry has also been reported (Kuiper et al., 1998; Mitchell and Dunnavan, 1998). Precise mechanisms of β-agonist action vary with its interrelationships with animal species, sex, dosage, or duration of treatment (Dalrymple et al., 1984; Buyse et al., 1991; Reeds and Mersmann, 1991; Wellenreiter, 1991). Recently, dietary conditions have been shown to affect growth performance in β-agonist-fed animals (Reeds and Mersmann, 1991; Hamano et al., 1994, 1998b). Furthermore, the effect on chicken performance seems to be weaker than that on mammals (Wellenreiter, 1991). Thus, in order to maintain the maximal growth performance of chickens that receive β-agonist, nutritional factors should be considered.

The role of vitamins in β-adrenergic response of tissues is obscure. A previous study found that the lipolytic effect of a β-agonist, clenbuterol, monitored by plasma NEFA was elicited by supplementation with thiamin (Hamano et al., 1999). We have further focused on the role of lipoic acid (ALA), classified as a B vitamin and an antioxidant, in the metabolic response of chickens to β-agonist. ALA exists endogenously in the tissues and acts as a cofactor in enzyme systems involved in the oxidative decarboxylation of α-keto acids (Christensen, 1983). Recent studies indicate that exogenous ALA treatment improves energy utilization in skeletal muscle in several pathological states. Glucose transport in insulin-resistant skeletal muscle of obese rats or streptozotocine-diabetic rats was stimulated by ALA supplementation (Jacob et al., 1996; Henriksen et al., 1997; Streeper et al., 1997). This vitamin is also reported to affect lipid metabolism and thyroid hormone metabolism (Ivanov, 1974; Segermann et al., 1991).

A previous study found that the administration of ALA led to a slight reduction in adipose tissue accretion, increased hepatic oxygen consumption, and stimulated glucose mobilization during continuous infusion of isoproterenol (Hamano et al., 1998a). However, this elevated plasma glucose concentration seemed to mask the effect on plasma NEFA. In fact, plasma NEFA as an index of lipolysis was markedly reduced by isoproterenol infusion, regardless of ALA administration. Thus, the ALA effect on lipolytic response to β-agonist may be confirmed in the state of a steady plasma glucose level. As a further experimental condition, we used a single injection of isoproterenol because the procedure causes only a transient increase in plasma glucose, which rapidly returns to the basal level. Therefore, the aim of this study was to examine the effects of ALA administration on plasma metabolites, and lipolytic response to isoproterenol injection when plasma glucose did not affect plasma NEFA level.

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Table 1. Effects of lipoic acid (ALA) on plasma metabolites in broiler chickens

Plasma metabolites[1]	Glucose (mg/100 mL)	NEFA (μEq/L)	Triglyceride (mg/100 mL)	Total cholesterol (mg/100 mL)
Control	197.9±6.3	560±44	31.1±1.4	81.7±4.8
ALA	196.4±6.6	544±43	42.9±5.8	72.3±4.6*

[1] Values represent means with SEM (n=8); * $p<0.05$.

MATERIALS AND METHODS

Day-old 52 female broiler chicks (*Ross*) were housed in battery brooders maintained at 33℃ and fed a commercial starter diet containing 23% CP and 3,200 kcal of ME per kg until 3 weeks of age. Thereafter, the diet provided was switched to a commercial finisher diet containing 18% CP and 3,100 kcal of ME per kg. Diets and water were provided *ad libitum* throughout the experimental period. The temperature in the battery brooders with continuous lighting was reduced by 2℃ per week and finally was maintained at 25℃. At 3 d of age, chicks were divided randomly into 4 groups of 13 birds and each group was separately housed in the battery brooders. Two groups were assigned for control, and two to the ALA treatment group. ALA (DL-thioctic acid, Kanto Chemical, Tokyo, Japan) was administered with food at the level of 50 mg/kg and provided over 24 d. This dose level was used because a previous study had found it produced significant metabolic effects on hepatic oxygen consumption and plasma glucose in response to continuous infusion of isoproterenol (Hamano et al., 1998a).

At 27 d of age, 4 chickens were randomly selected from each group for blood sampling (n=8) and used for isoproterenol challenge. After blood was drawn from a wing vein, the chickens were weighed and placed in restrainers. A medical catheter (o.d.=0.68 mm, i.d.=0.48 mm; Nipro, Osaka, Japan) was inserted into a wing vein under local anesthesia. Two blood samples were taken with a heparinized syringe before injection of isoproterenol (Sigma, St. Louis, MO, USA). The first was drawn 10 min before injection time, and β-agonist was injected immediately after the second. Isoproterenol dissolved in 0.9% saline was infused into the wing vein at the dosage of 2 mg per kg BW (as solution volume of 1.0 mL/kg BW), and blood samples (1 mL) were taken at 10, 20, 30, 60, 90, 120, and 180 min. Basal concentrations of plasma metabolites in the operated chickens were unchanged as compared with their levels before surgery.

Plasma obtained from centrifuged blood samples (2,000 g) was stored at -20℃ until chemical analysis for plasma metabolites. Concentrations of plasma metabolites were colorimetrically determined using enzymatic assay kits for glucose (Kyowa Medex, Tokyo, Japan), NEFA (Kyowa Medex, Tokyo, Japan), total cholesterol (Kyowa Medex, Tokyo, Japan), and triglyceride (Sanko Chemical, Tokyo, Japan). Students t-test was used to compare the mean values of plasma metabolites between control and ALA-fed groups. Data from the isoproterenol challenge were analyzed using two-way ANOVA taking into account as main factors the post-injection time and ALA treatment (StatView, Abacus Concepts Inc., Berkley, CA, USA). The mean values (change from basal values) between control and ALA-fed groups at each post-injection time were compared using t-test, when significant effect of ALA on the change in plasma metabolite with isoproterenol challenge was detected.

RESULTS AND DISCUSSION

Table 1 shows the long-term effects of ALA on plasma metabolites in broilers. ALA is a cofactor in the enzyme complexes that catalyze the oxidative decarboxylation of α-keto acids (e.g., α-ketoglutarate dehydrogenase and pyruvate dehydrogenase). This is associated especially with glucose metabolism. Recent studies demonstrated that ALA stimulates glucose uptake in skeletal muscle and reduces hyperglycemia (Jacob et al., 1996; Streeper et al., 1997). However, in normal chickens, dietary ALA at the level of 50 mg/kg did not affect plasma glucose concentration.

Segermann et al. (1991) noted that the serum triglyceride decreased without change in serum glucose in the ALA-treated rats, and a reduction in tissue triglyceride due to the administration of ALA was also observed in calculogenic rats (Jayanthi and Varalakshmi, 1992). These effects are dependent on hepatic lipogenesis because ALA supplementation seems to inhibit acetyl-CoA carboxylase activity in the rat liver (Zempleni et al., 1997). In chickens, a previous study indicated that the increase in hepatic oxygen consumption seems to be responsible for a fat accretion reduction in 4-week-old broilers that had received ALA at the level of 50 mg/kg diet (Hamano et al., 1998a). However, plasma triglyceride concentration in the ALA-fed chickens was unchanged in this study. Likewise, dietary ALA had no effect on the plasma NEFA level, estimated as lipolytic response of adipose tissue. Thus, dietary ALA administration alone did not elicit significant changes in neutral fat transport or fatty acid mobilization, as monitored by plasma concentrations of the metabolites.

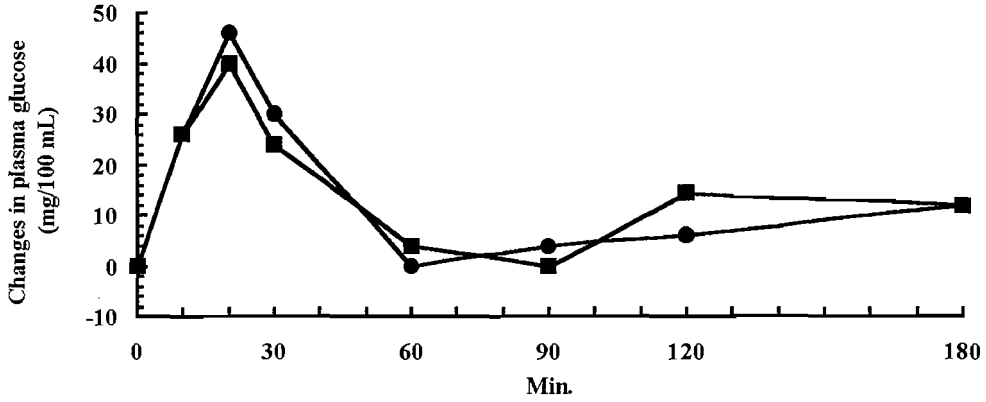

Figure 1. Effects of isoproterenol injection on plasma glucose in broilers with or without ALA treatment. Values represent means for 8 observations (●, control; ■, ALA). Pooled SEM=3.6, control; 4.6, ALA

On the other hand, dietary ALA reduced ($p<0.05$) plasma total cholesterol by 25%. Ivanov (1974) reported that ALA administered to rabbits with experimental atherosclerosis markedly reduced plasma cholesterol and β-lipoproteins. In rats, serum cholesterol decreased markedly when both ALA and thyroxine were administered (Segermann et al., 1991). The present result might be attributable to the decreased activity of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, a key enzyme in the biosynthesis of cholesterol. However, thyroid hormone administration in hypothyroidism has been reported to decrease plasma cholesterol to normal but increase HMG-CoA activity in the liver (Field et al., 1986; Day et al., 1989). Even though the ALA treatment inhibited cholesterol synthesis, decreased plasma cholesterol in the treated chickens would be due merely to reduced lipoprotein transport. In the present study, whether the reduced plasma total cholesterol resulted from a stimulated oxidative process in the liver that is similar to the observation reported by Ivanov (1974) is unclear.

The present lack of alteration of plasma metabolites, except for total cholesterol, might be associated with dose level of ALA, although the dietary level used has been confirmed to be effective in the tissue response for chickens (Hamano et al., 1998a). Metabolic response of chickens to ALA supplementation may also be dependent on ALA absorption and transport from gastrointestinal tract to tissues for utilization in the reduced form. Most exogenous ALA that was orally administered is absorbed quantitatively (Peter and Borbe, 1995). The intraperitoneal injection of ALA increased its plasma concentration in rats (Zempleni et al., 1997), but about 80% of an intraperitoneal dose of ALA was eliminated within 24 h (Spence and McCormick, 1976). The rate of reduction in plasma ALA concentration (half-life) when administered orally seems to be similar to that when injected intraperitoneally (Peter and Borbe, 1995). Thus, the lack of metabolic response to ALA was probably dependent on the dose levels rather than the route of administration in this study. Administration of more than 50 mg/kg may be necessary for a beneficial effect on plasma metabolites or energy redistribution in normal chickens.

Figure 1 shows the effect of isoproterenol injection on the plasma glucose level in chickens with or without ALA treatment. Plasma glucose, as compared with basal values, rapidly increased ($p<0.001$) in both groups following isoproterenol injection. At 20 min after injection, the response was maximal. The increase in blood glucose due to the isoproterenol injection is, in general, transient and small, and must be attributable to the stimulated glycogenolysis (Yang and McElligott, 1989). In this study, the glucose concentration had fallen back to the basal level at 60 min after injection. However, no significant relation between ALA and isoproterenol was observed in ANOVA ($p>0.05$). ALA is reported to stimulate glucose uptake in insulin-resistant rat skeletal muscle (Jacob et al., 1996) and to reduce blood glucose level in hyperglycemia with an increase in glucose transporter 4 protein in diabetic rats (Khamaisi et al., 1997), although this response was not associated with glucose transporter 4 mRNA levels (Khamaisi et al., 1997). In contrast, when chickens were treated with a continuous infusion of isoproterenol, this drug-induced glucose release was surprisingly stimulated by ALA supplementation (Hamano et al., 1998a). Thus, the transient glucose mobilization in the liver when treated by single injection of isoproterenol was not acutely affected by the dietary ALA treatment.

Figure 2 shows the effects of isoproterenol injection on plasma NEFA in broilers treated with or without ALA. In contrast to glucose, plasma NEFA

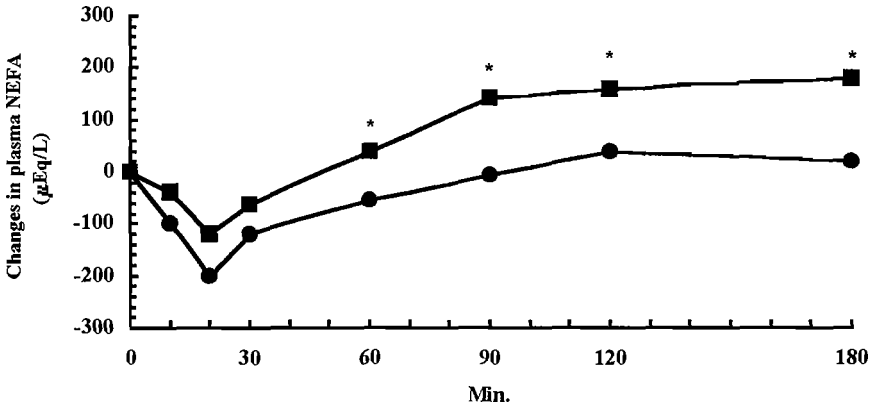

Figure 2. Effects of isoproterenol injection on plasma NEFA in broilers with or without ALA treatment. Values represent means for 8 observations (●, control; ■, ALA). Pooled SEM=18, control; 26, ALA
* Significant difference between control and ALA-treated chickens at each time after injection ($p<0.05$).

was rapidly lowered ($p<0.001$) by isoproterenol stimulation. The depressed NEFA may be associated with the increased glucose level, because the maximal response occurred at 20 min after injection. Then, NEFA returned to the basal level almost immediately, and in ALA-treated chickens exceeded the basal level furthermore ($p<0.001$). This enhanced NEFA was significantly higher ($p<0.05$) than in controls during 60 to 180 min post-injection. When chickens received a continuous infusion of isoproterenol, plasma NEFA continued to fall with a marked increase in glucose until 120 min post-injection (Hamano et al., 1998a). Chicken adipocytes were reported to be insensitive to epinephrine *in vitro* (Weppelman, 1984), and this insensitivity seems to correlate with the proportion of a subclass for β-receptors, β_1 or β_2, existing on tissue cells (Weppelman, 1984; Wellenreiter, 1991; Kim et al., 1992). A combination of a β-agonist, clenbuterol, and thiamin administered to broiler chicks also resulted in enhancement of NEFA by about 70%, as previously reported (Hamano et al., 1999). Although isoproterenol injection in cows has been shown to increase both plasma NEFA and glucose simultaneously and rapidly (Chilliard and Ottou, 1995), in this study, the isoproterenol-induced fatty acid mobilization was confirmed only in the ALA-fed chickens after plasma glucose stimulated by the β-agonist recovered to the basal level. Thus, the present finding suggests that ALA affected adipose tissue lipolysis in the β-adrenergic response in chickens.

Zempleni et al. (1997) reported that the administration of ALA reduced the activities of pyruvate carboxylase and β-methylcrotonyl-CoA carboxylase in the rat liver. β-Methylcrotonyl-CoA carboxylase is related to leucine degradation resulting in acetoacetete production. They suggest that ALA prevents biotin from being incorporated into one or more of the holocarboxylase. The administration of ketone bodies decreased catecholamine-stimulated lipolysis in adipocytes (Larsen et al., 1983). As a possible interrelationship with β-adrenergic regulation, the exogenous ALA might affect not only glucose metabolism but also fatty acid oxidation by reducing the ketone body production due to β-adrenergic stimulation. Catecholamine stimulation has been reported to increase hepatic ketogenesis in rats (Kosugi et al., 1983). Isoproterenol also enhanced ketogenesis from caprinoate in hepatocytes (Schulze et al., 1986). Whether ALA or isoproterenol affected plasma ketone body production is unclear, but the result of isoproterenol-stimulated fatty acid mobilization would be involved indirectly in changes in ALA-related enzyme activities.

The effect of isoproterenol injection on plasma triglyceride in chickens treated with or without ALA is shown in figure 3. Triglyceride concentration was markedly reduced ($p<0.001$) following isoproterenol injection. The maximal change was found at 10 min after injection and tended to be greater in the ALA-fed chickens than in controls. When chickens received a continuous infusion of isoproterenol, no difference in the reduction of triglyceride between control and ALA treatment was observed (Hamano et al., 1998a). The long-term administration of β-agonist is reported to reduce blood triglyceride or lipoprotein level in animals including chickens (Buyse et al., 1991; Wellenreiter, 1991). Compared to mammals, a decrease in adipose tissue accretion of the β-agonist-fed chickens seems to be attributable mostly to inhibition of fat synthesis and acetyl-CoA carboxylase activity (Yang and McElligott, 1989; Wellenreiter, 1991). However, the isoproterenol-induced decrease in plasma triglyceride possessed no significant interrelationship with the ALA supplementation ($p>0.05$).

Over all, long-term treatment with ALA alone decreased plasma cholesterol without altering plasma glucose, NEFA or triglyceride. This finding might be

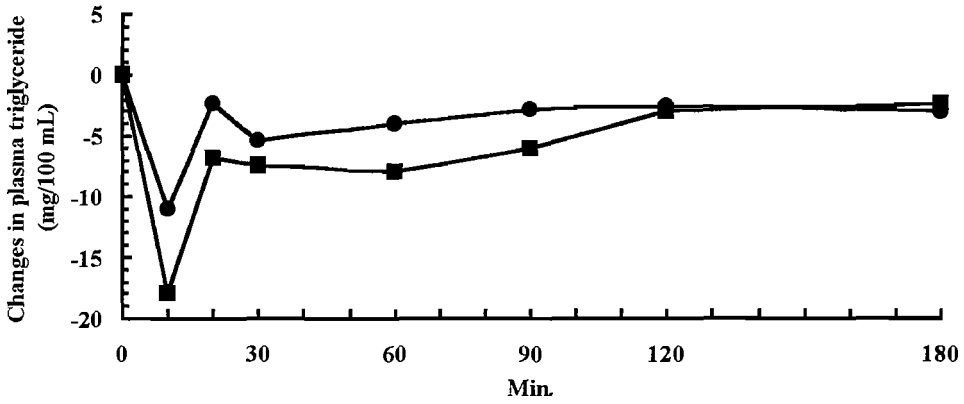

Figure 3. Effects of isoproterenol injection on plasma triglyceride in broilers with or without ALA treatment. Values represent means for 8 observations (●, control; ■, ALA). Pooled SEM=0.6, control; 1.3, ALA

associated with the dose level, and the lipolytic response of a β-agonist may be refractory in chickens, but ALA aided and improved it. Therefore, the present study suggested that the dietary administration of ALA was involved in fatty acid mobilization in response to β-agonist in broilers.

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