ELSEVIER

European Journal of Pharmacology 332 (1997) 215–218

ejp

Short communication

Chronic treatment with BRL 35135 potentiates the action of insulin on lipid metabolism

Kirsi A. Virtanen *, Juha Rouru, Virve Hänninen, Eriika Savontaus, Taina Rouvari, Tomi Teirmaa, Markku Koulu, Risto Huupponen

Department of Pharmacology and Clinical Pharmacology, University of Turku, Kiinanmyllynkatu 10, FIN-20520 Turku, Finland

Received 9 May 1997; accepted 20 June 1997

Abstract

The effects of a β_3-adrenoceptor agonist on insulin-induced changes in lipid metabolism were studied in obese male Zucker (*fa/fa*) rats during euglycaemic clamp. Rats were treated with BRL 35135 (*R**,*R**-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)-ethyl-amino]-propyl]-phenoxyacetate hydrobromide) (0.5 mg/kg per day in drinking water) for three weeks before an euglycaemic hyperinsulinaemic clamp was performed. Insulin infusion lowered serum non-esterified fatty acids and plasma glycerol more efficiently in BRL 35135-treated than in control rats although plasma insulin remained significantly lower in the BRL 35135-treated than in the control rats during the clamp. In conclusion, chronic treatment with BRL 35135 potentiates the effect of insulin on lipid metabolism. © 1997 Elsevier Science B.V.

Keywords: BRL 35135; β_3-adrenoceptor agonism; Zucker (*fa/fa*) rat; Insulin sensitivity; Non-esterified fatty acid; Euglycaemic hyperinsulinaemic clamp

1. Introduction

BRL 35135 (*R**,*R**-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)-ethyl-amino]-propyl]-phenoxyacetate hydrobromide) is a selective β_3-adrenoceptor agonist which activates brown adipose tissue thermogenesis in rodents (Arch et al., 1984). Treatment with β_3-adrenoceptor agonists increases the metabolic rate, energy expenditure and reduces body lipid content and weight gain and improves insulin sensitivity (Arch et al., 1984; Smith et al., 1990). Acutely BRL 35135 stimulates lipolysis (Arch et al., 1984) which increases serum non-esterified fatty acid concentration in rodents (Liu and Stock, 1995). Released fatty acids in turn stimulate thermogenesis of brown adipose tissue (Arch et al., 1984).

Lipid metabolism has an important impact on tissue glucose handling through glucose-free fatty acid cycle (Randle et al., 1963). Normally insulin suppresses plasma non-esterified fatty acid concentration (Randle et al., 1963) and conversely, impaired insulin sensitivity is associated with elevation of plasma non-esterified fatty acids (Ferrannini et al., 1983). In this study we investigated whether treatment with BRL 35135, a lipolytic agent, modulates the effect of insulin on plasma lipids in insulin resistant Zucker (*fa/fa*) rats.

2. Materials and methods

2.1. Animals

Obese male Zucker (*fa/fa*) rats were purchased from IFFA Credo (L'Arbresle, France). The animals were individually housed and maintained under a constant light–dark cycle (lights on 06.00). They had free access to water and to normal laboratory rat chow (R36, Lactam, Stockholm, Sweden) containing 12.6 MJ/kg metabolizable energy.

2.2. Drugs

BRL 35135 was a generous gift from SmithKline Beecham Pharmaceuticals (Guildford, UK). Drug stock solutions were freshly prepared in water. Human Insulin

* Correspondence author. Tel.: (358-2) 333-7517; Fax: (358-2) 333-7216; e-mail: kirsi.virtanen@utu.fi

PII S0014-2999(97)01104-7

(Actrapid®, 100 IU/ml) used in euglycaemic clamp was manufactured by Novo Nordisk (Bagsværd, Denmark).

2.3. Experimental protocol

The rats were 9–10 weeks old when they were divided in two groups one of which ($n = 6$) received 0.5 mg/kg BRL 35135 (Santti et al., 1994) per day in drinking water whereas the other group ($n = 6$) received tap water only. The concentration of BRL 35135 in the drinking water was regularly adjusted to maintain the correct daily dose. The groups were matched with basal body weight and food intake at the beginning of the experiment. Body weights were measured every other day. After three weeks, an euglycaemic hyperinsulinaemic clamp was performed. Food was withdrawn 24 h before the clamp study in both groups. Water and drugs were available until the beginning of anesthesia.

2.4. Euglycaemic clamp technique

All clamp studies were performed between 08.00 a.m. and 01.00 p.m. on anesthetized rats (pentobarbital; Mebunat® 60 mg/ml (Orion, Espoo, Finland); 50 mg/kg, i.p.) as described earlier by Leturque et al. (1984). A basal blood sample (1.0–1.5 ml) was taken before glucose and insulin infusions for determinations of plasma glucose, insulin, lactate, triglycerides, cholesterol, glycerol, leptin and serum non-esterified fatty acids. Another sample was collected during clamp study at 120 min (steady state) for the determinations given above.

2.5. Analytical methods

Blood samples were immediately centrifuged at 4°C (unless used for blood glucose determination during the clamp) and the plasma was kept frozen at −70°C until analyzed. Plasma glucose, lactate and cholesterol were determined with the Analox GM7 measuring device (Analox Instruments, London, UK). The glucose concentration in whole blood during the clamp was measured with a Glucoscot device (DIC, Kyoto, Japan). Plasma insulin was measured by Insulin RIA kit (Kabi Pharmacia Diagnostics, Uppsala, Sweden). Plasma triglycerides were determined immediately prior freezing the plasma by esterase–glycerokinase–glycerol phosphatase oxidase reaction with strip test (Reflotron®, Boehringer-Mannheim, Mannheim, Germany). Serum non-esterified fatty acids were measured by enzymatic method with colorimetry (NEFA C, Wako, Neuss, Germany). Plasma glycerol was determined colorimetrically (Randox, Antrim, UK). Plasma leptin was measured by Rat Leptin RIA Kit (Linco Research, St. Charles, MO, USA). Metabolic index (i.e., mean glucose infusion rate per body weight and minute) was calculated during the last 50 min of the clamp study in the steady state.

2.6. Statistical analysis

Student's *t*-test was used to compare two means with each other. Two-way analysis of variance (ANOVA), allowing testing of treatment effect (active drug vs. vehicle), effect of hyperinsulinemia (basal values vs. values at the end of clamp) and their interaction was performed when appropriate using SYSTAT software (Systat, Evanston, IL, USA). A significant interaction term denotes that the change from the basal state to the hyperinsulinemic state was different in the BRL 35135-treated and control rats. Values are means with standard errors of the mean. A *P*-value less than 0.05 was considered statistically significant.

3. Results

3.1. Basal values

When compared with the control treatment, BRL 35135 significantly increased the mean daily fluid intake by 17% ($P = 0.008$, *t*-test) and reduced weight gain by 29% ($P = 0.023$) during the three week period. Plasma glucose, lactate and cholesterol concentrations did not differ between the groups (Table 1). Similarly, BRL 35135 had no

Table 1
Plasma glucose, insulin, lactate, cholesterol, triglycerides and leptin at the basal state and during euglycaemic hyperinsulinaemic clamp in the control and BRL 35135-treated (three weeks with 0.5 mg/kg per day in the drinking water) obese Zucker (*fa/fa*) rats

	Control	BRL 35135-treated
Basal state		
Glucose	10.0 ± 0.4	8.7 ± 0.6
Insulin	135 ± 12.3	134 ± 19.6
Lactate	3.2 ± 0.4	2.6 ± 0.3
Triglycerides	3.0 ± 0.4	2.6 ± 0.4
Cholesterol	4.4 ± 0.3	3.9 ± 0.3
Leptin	86.2 ± 11.0	53.8 ± 6.2 [a]
Hyperinsulinemic state		
Glucose	9.8 ± 0.6	9.9 ± 0.5
Insulin	1209 ± 203 [b]	529 ± 57 [b,c]
Lactate	5.6 ± 0.9 [d]	3.8 ± 0.4 [d]
Triglycerides	2.8 ± 0.5	2.3 ± 0.3
Cholesterol	3.5 ± 0.2 [d]	3.0 ± 0.1 [e]
Leptin	108.3 ± 12.6	63.8 ± 12.4 [f]

Values are means ± S.E.M., $n = 6$ in both groups. The results are expressed as mmol/l, except for insulin (μU/ml) and leptin (ng/ml).
[a] Lower than in the respective control group, $P = 0.05$ (*t*-test, $n = 4$ in both groups).
[b] Higher than at basal state, $P < 0.001$.
[c] Lower than in the respective control group, $P = 0.027$.
[d] Higher than at basal state, $P = 0.003$.
[e] Lower than at basal state, $P = 0.02$.
[f] Lower than in the respective control group, $P = 0.04$ (*t*-test, $n = 4$ in both groups).

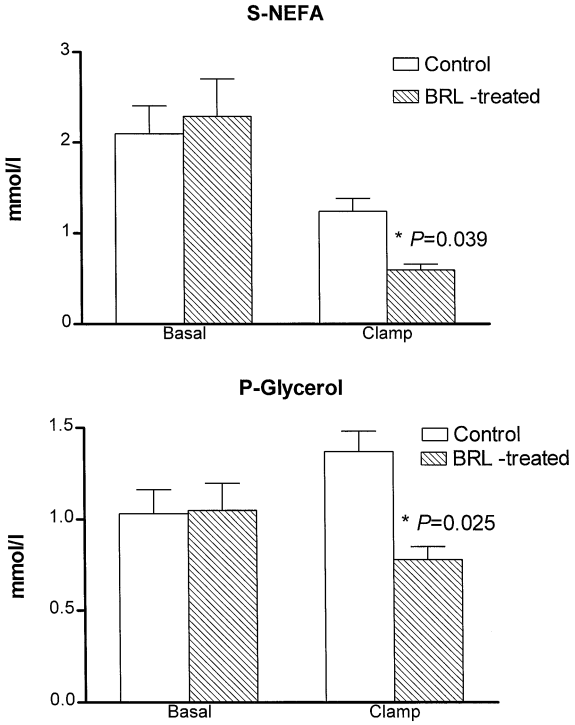

Fig. 1. The effect of 3 weeks treatment with BRL 35135 on plasma glycerol and serum non-esterified fatty acids (NEFA) at basal state and during euglycaemic hyperinsulinaemic clamp in obese Zucker rats. * = the change from the basal level significantly different from the control group (two-way ANOVA). $n = 6$ in both groups, mean ± S.E.M. are given.

effect on plasma insulin, triglycerides, glycerol or serum non-esterified fatty acids at the basal state but the β_3-adrenoceptor agonist reduced plasma leptin concentration (Table 1).

3.2. Euglycaemic hyperinsulinaemic clamp study

The euglycaemic clamp was performed at equivalent glucose levels in both groups (Table 1). At basal state, plasma insulin was similar in the control and intervention group ($P = 0.96$, *t*-test) whereas at the end of insulin infusion, plasma insulin concentrations were 2.2-fold higher in control than in BRL 35135-treated animals. The change in plasma insulin from the basal level was different between the groups (interaction $F = 6.92$, $P = 0.027$, ANOVA). Serum non-esterified fatty acids were powerfully reduced by insulin infusion ($F = 37.2$, $P < 0.001$, ANOVA) the effect being more pronounced in the BRL 35135 than in the control group (interaction $F = 5.6$, $P = 0.039$, ANOVA, Fig. 1). Similarly, plasma glycerol was reduced in BRL 35135-treated but not in control animals in response to insulin infusion (interaction $F = 6.9$, $P = 0.025$, ANOVA, Fig. 1). Plasma lactate concentrations increased by insulin infusion ($F = 15.58$, $P = 0.003$; ANOVA) in both groups. Plasma cholesterol decreased in response to insulin ($F = 7.72$, $P = 0.02$, ANOVA) but again there was no difference between the groups in this respect. Plasma triglyserides were not affected by insulin infusion or BRL 35135-treatment. Plasma leptin was reduced by BRL 35135 treatment ($F = 8.00$, $P = 0.03$, ANOVA); it tended to be elevated in a similar manner by insulin infusion in both groups ($F = 5.11$, $P = 0.065$, ANOVA; Table 1). When glucose uptake (M) was related to the steady state plasma insulin concentration (I), BRL 35135 caused on average a 44% increase in M/I ratio, but the difference between control and BRL 35135-treated Zucker rats was not statistically significant ($P = 0.143$, *t*-test).

4. Discussion

The serum fatty acids suppressing effect of insulin is impaired in obesity (Coppack et al., 1992). The most important finding in our study was that BRL 35135-treatment significantly enhanced the serum non-esterified fatty acid-lowering effect of insulin. The potentiation of the insulin action was powerful enough to be significant even when steady state plasma insulin level during the clamp was lower in the BRL 35135-treated group. In accordance with its β_3-adrenoceptor stimulating effect, acute administration of BRL 35135 increases plasma fatty acid concentration (Liu and Stock, 1995). During chronic treatment, however, fatty acid concentration may not change (Liu and Stock, 1995) or may even be lowered (Arbeeny et al., 1995). Plasma glycerol was reduced during insulin infusion in the BRL 35135-treated group when compared to control animals which is consistent with a potentiation of the antilipolytic effect of insulin by chronic β_3-adrenoceptor agonist treatment.

Hyperinsulinaemia during an euglycaemic clamp inhibits lipolysis and decreases the plasma non-esterified fatty acid concentration (Ferrannini et al., 1983) providing a measure of insulin action on adipose tissue. In the present study the serum fatty acid level was suppressed to a significantly lower level by hyperinsulinaemia in BRL 35135-treated rats. This finding suggests that insulin had a better ability to inhibit lipolysis in BRL 35135-treated rats and consequently, an increased insulin sensitivity at the level of adipose tissue. Interestingly, treatment with BRL 35135 reduced plasma leptin concentration and insulin infusion tended to elevate it which are in accordance with in vitro studies where incubation of adipocytes with insulin concentration-dependently increased leptin release which could be inhibited by a β_3-adrenoceptor agonist (Gettys et al., 1996).

It is puzzling how a β_3-adrenoceptor agonist acutely causing lipolysis augments the fatty acid-lowering effect of insulin. Mechanisms leading to reduction in serum fatty acid concentration may include increased tissue uptake of fatty acids, especially to brown adipose tissue. Hashimoto et al. (1996) showed that mesenterial fat was dose-depen-

dently decreased during BRL 35135-treatment in Zucker (*fa/fa*) rats supporting the lipolytic effect in white adipose tissue. Indeed, chronic treatment of obese mice with BRL 35135 resulted in a shift of the triglyceride storage from white adipose tissue to brown adipose tissue (Arbeeny et al., 1995) suggesting a net flux of fatty acids to brown adipose tissue. Since BRL 35135 powerfully stimulates glucose utilization in brown adipose tissue (Liu and Stock, 1995) and insulin increases both glucose uptake in adipose tissue and the activity of lipoprotein lipase (Robinson, 1963), the esterification capacity of fatty acids to triglyserides is increased in brown adipose tissue during concomitant insulin and β_3-adrenergic agonist administration. Therefore, it can be expected that due to the powerful stimulation of brown adipose tissue thermogenesis by a β_3-adrenoceptor agonist, the fuel demand of the tissue increases and available fatty acids are efficiently used by this tissue.

During the clamp, plasma insulin was 2.2-times lower in BRL 35135-treated than in control rats despite identical insulin infusion rates, identical glucose levels during the clamp and comparable basal insulin concentrations in both groups. The reason of this finding remains speculative. BRL 35135 may have augmented the suppressing effect of exogenous insulin in the pancreas or the weight decrease may have upregulated the number of insulin receptors on the cell membrane (Rochet et al., 1988) resulting in increased clearance of the hormone. Finally, since hepatic clearance of insulin is in an inverse manner proportional to the fatty acid concentration in the portal vein (Svedberg et al., 1991) the reduction in fatty acid concentration may have increased the clearance of insulin.

In conclusion, chronic treatment with BRL 35135 markedly enhances the serum fatty acid and plasma glycerol lowering effect of insulin. This may have important consequences for carbohydrate metabolism and contribute to increased insulin sensitivity during β_3-adrenoceptor agonist treatment.

Acknowledgements

This study was financially supported by TEKES (The Centre of Technological Development, Helsinki, Finland). We are grateful to Ms. Taina Lehti, Ms. Elina Kahra and Ms. Raija Kaartosalmi for their skilful assistance.

References

Arbeeny, C.M., Meyers, D.S., Hillyer, D.E., Bergquist, K.E., 1995. Metabolic alterations associated with the antidiabetic effect of β_3-adrenergic receptor agonist in obese mice. Am. J. Physiol. 268 (Endocrinol. Metab. 31), E678–E684.

Arch, J.R.S., Ainsworth, A.T., Cawthorne, M.A., Piercy, V., Sennitt, M.V., Thody, V.E., Wilson, C., Wilson, S., 1984. Atypical β-adrenoceptor on brown adipocytes as target for anti-obesity drugs. Nature 309, 163–165.

Coppack, S.W., Evans, R.D., Fisher, R.M., Frayn, K.N., Gibbons, G.F., Humphreys, S.M., Kirk, M.L., Potts, J.L., Hockaday, T.D., 1992. Adipose tissue metabolism in obesity: Lipase action in vivo before and after a mixed meal. Metabolism 41, 264–272.

Ferrannini, E., Barrett, E.J., Bevilacqua, S., DeFronzo, R.A., 1983. Effect of fatty acids on glucose production and utilization in man. J. Clin. Invest. 72, 1737–1747.

Gettys, T.H., Harkness, P.J., Watson, P.M., 1996. The β_3-adrenergic receptor inhibits insulin-stimulated leptin secretion from isolated rat adipocytes. Endocrinology 137, 4054–4057.

Hashimoto, K., Nagao, Y., Ida, K., Takeda, M., Murakami, N., Kato, K., Mizota, M., 1996. Improvement of metabolic disorders and visceral fat obesity by the β_3-adrenoceptor agonist (R^*,R^*)-(±)-methyl-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]-phenoxyacetate hydrobromide (BRL 35135A) in genetically obese rodents. Biochem. Pharmacol. 52, 1529–1535.

Leturque, A., Burnol, A.F., Ferré, P., Girard, J., 1984. Pregnancy-induced insulin resistance in the rat: Assessment by glucose clamp technique. Am. J. Physiol. 246 (Endocrinol. Metab. 9), E25–E31.

Liu, Y.-L., Stock, M.J., 1995. Acute effects of the β_3-adrenoceptor agonist, BRL 35135, on tissue glucose utilization. Br. J. Pharmacol. 114, 888–894.

Randle, P.J., Hales, C.N., Garland, P.B., Newsholme, E.A., 1963. The glucose fatty-acid cycle, its role in insulin sensitivity and the metabolic disturbances of diabetes mellitus. Lancet 1, 785–789.

Robinson, D.S., 1963. The clearing factor lipase and its action in the transport of fatty acids between blood and the tissues. Adv. Lipid Res. 1, 133–182.

Rochet, N., Tanti, J.F., Gremeaux, T., Van Obberghen, E., Marchand-Brustel, Y., 1988. Effect of the thermogenic agent BRL 26830A on insulin receptors in obese mice. Am. J. Physiol. 255, E101–E109.

Santti, E., Huupponen, R., Rouru, J., Hänninen, V., Pesonen, U., Jhanwar-Uniyal, M., Koulu, M., 1994. Potentiation of the antiobesity effect of the selective β_3-adrenoceptor agonist BRL 35135 in obese Zucker rats by exercise. Br. J. Pharmacol. 113, 1231–1236.

Smith, S.A., Sennitt, M.V., Cawthorne, M.A., 1990. BRL 35135: An orally active antihyperglycaemic agent with weight reducing effects. In: Bailey, C.J., Flatt, P.R. (Eds.), New Anti-Diabetic Drugs. Smith-Gordon, London, pp. 177–189.

Svedberg, J., Strömblad, G., Wirth, A., Smith, U., Björntorp, P., 1991. Fatty acids in the portal vein of the rat regulate hepatic insulin clearance. J. Clin. Invest. 88, 2054–2058.