# A new potentiator of insulin action

## Post-receptor activation in vitro

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A new potentiator of insulin action, 5-[4-(1-methylcyclohexylmethoxy)-benzyl]thiazolidine-2,4-dione, was tested for activation of insulin action in vitro. The agent (50 mg.kg$^{-1}$.day$^{-1}$) was orally administered to rats for 14 days and adipocytes from treated rats were used to assess insulin-binding, glucose uptake and glucose oxidation. In obese rats, the agent increased glucose uptake and oxidation without any change in insulin binding, whereas in lean or streptozotocin-treated rats it failed to increase glucose metabolism. Fat tissues were cultured with the agent for 24 h and were tested for insulin action. In the presence of insulin (10 ng/ml) in the culture media, the agent increased glucose oxidation in these cells without any change in insulin binding. However, without insulin in the culture media the agent did not increase glucose oxidation. Thus, the agent appeared to potentiate insulin action at the post receptor process.

*Insulin* *Receptor* *Diabetes* *Obesity* *Insulin resistance*

## 1. INTRODUCTION

Various agents including sulfonyl urea and biguanides have been used for alleviation of glucose intolerance in diabetes mellitus. These agents appeared to increase glucose metabolism by extrapancreatic or pancreatic effects [1,2]. Furthermore, these agents proved to potentiate insulin action in vivo [3] and in vitro [4] when insulin is present. Some of these agents are reported to increase insulin receptor capacity which may contribute to potentiate insulin action [5,6].

We report here that a new chemical agent, 5-[4-(1-methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (ADD-3878 or ADD) can potentiate insulin action in rat adipocytes without any change in insulin receptors and that this agent itself does not possess insulin-like activity. This agent may be useful for treatment of Type-II diabetes mellitus with increased insulin resistance and hyperinsulinemia by potentiating insulin action.

## 2. MATERIALS AND METHODS

5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (ADD-3878) was chemically synthesized (fig.1) and was supplied by Takeda Chemical Industries (Osaka). Porcine insulin (Lot 1FJ91, 26.2 units/mg) was kindly supplied by Eli Lilly. $Na^{125}I$ was purchased from New England Nuclear and culture medium TCM 199 from Gibco. Bovine serum albumin (fraction V) was purchased from Armour Pharmaceuticals, collagenase from Worthington Biochemicals, 2-deoxy-[1-$^{14}$C]glucose, L-[$^{14}$C]glucose and D-[1-$^{14}$C]-glucose from New England Nuclear.

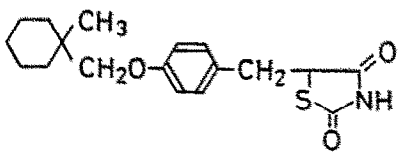


Fig.1. Structure of ADD-3878.



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ADD-3878 (50 mg.kg$^{-1}$.day$^{-1}$) in saline was orally administered to 3 groups of Sprague-Dawley rats (lean young rats, 160–170 g, obese old rats, 390–415 g and (55 mg/kg) streptozotocin-treated rats, 170–185 g) for 14 days. The weight-matched controls were similarly treated with saline. Rats were then killed and adipocytes from epididymal tissues were isolated by collagenase and were used for insulin-binding and glucose metabolism studies. The methods of insulin-binding, glucose uptake and glucose oxidation studies were as in [7,8]. Insulin-binding was performed at 37°C for 45 min in the buffer containing bacitracin 50 mg/ml, pH 7.4. Iodination of insulin was performed as in [9] and the specific activity of the iodinated insulin was 100–150 $\mu$Ci/$\mu$g.

In order to directly determine in vitro action of ADD-3878 to potentiate insulin action, adipose tissues (about 10 mg/piece) were cultured with ADD-3878 in TCM 199 medium with 5% bovine serum albumin and 10 mM glucose, with and without insulin for 24 h at 37°C [10]. Then, isolated adipocytes from the tissues were tested for insulin-binding and glucose oxidation.

## 3. RESULTS AND DISCUSSION

Blood glucose and insulin values of rats after 14 days administration of ADD-3878 are shown in table 1. Plasma insulin values were decreased in ADD-treated obese rats by the administration of the agent, suggesting that insulin sensitivity was increased in obese rats treated with ADD.

Insulin-binding to isolated adipocytes from ADD-treated rats was comparable to that in non-treated rats as shown in table 1. Thus, administration of the agents did not affect insulin receptors.

In order to determine insulin action in these

Table 1

Characteristics of experimental animals[a]

| | Body weight[b] (g) | Plasma glucose[c] (mg/dl) | Plasma insulin[c] ($\mu$units/ml) | Bound insulin[e] (%) |
|---|---|---|---|---|
| Obese rats | | | | |
| ADD-treated ($n$ = 6) | 442 ± 14 | 164 ± 7 | 52 ± 3[d] | 4.1 ± 0.3 |
| Non-treated ($n$ = 6) | 444 ± 11 | 161 ± 6 | 71 ± 4 | 3.9 ± 0.4 |
| Lean rats | | | | |
| ADD-treated ($n$ = 6) | 212 ± 10 | 124 ± 5 | 32 ± 3 | 3.5 ± 0.3 |
| Non-treated ($n$ = 6) | 207 ± 8 | 126 ± 3 | 31 ± 4 | 3.3 ± 0.4 |
| Streptozotocin-treated rats | | | | |
| ADD-treated ($n$ = 4) | 176 ± 8 | 402 ± 12 | 17 ± 1 | 4.2 ± 0.4 |
| Non-treated ($n$ = 4) | 182 ± 7 | 410 ± 10 | 16 ± 1 | 4.3 ± 0.2 |

[a] All the values represent mean ± SEM

[b] Body weight represents the value on the day of experiment. Weight gain during 14 days treatment was comparable between ADD-treated and non-treated groups

[c] The values of plasma glucose and plasma insulin were the data of 9 a.m. on the day of experiment

[d] Difference between ADD-treated and non-treated was statistically significant ($p < 0.05$)

[e] Insulin-binding to $2 \times 10^5$ adipocytes from the rats after ADD-treatment or non-treatment for 14 days. Differences between treated and non-treated groups were not statistically significant. The values indicate % bound at a trace concentration

adipocytes from ADD-treated obese rats, glucose uptake and glucose oxidation studies were performed. Fig.2 shows significantly increased glucose uptake and oxidation in isolated adipocytes from ADD-treated obese rats.

Similar studies were performed on adipocytes from lean rats and streptozotocin-treated rats. Glucose oxidation was comparable in both ADD-treated and untreated streptozotocin-treated rats (fig.3), indicating that the presence of a certain amount of insulin in the plasma was necessary for the effect of the agent to appear, and that the agent did not potentiate insulin action in the normal rats without insulin resistance and hyperinsulinemia.

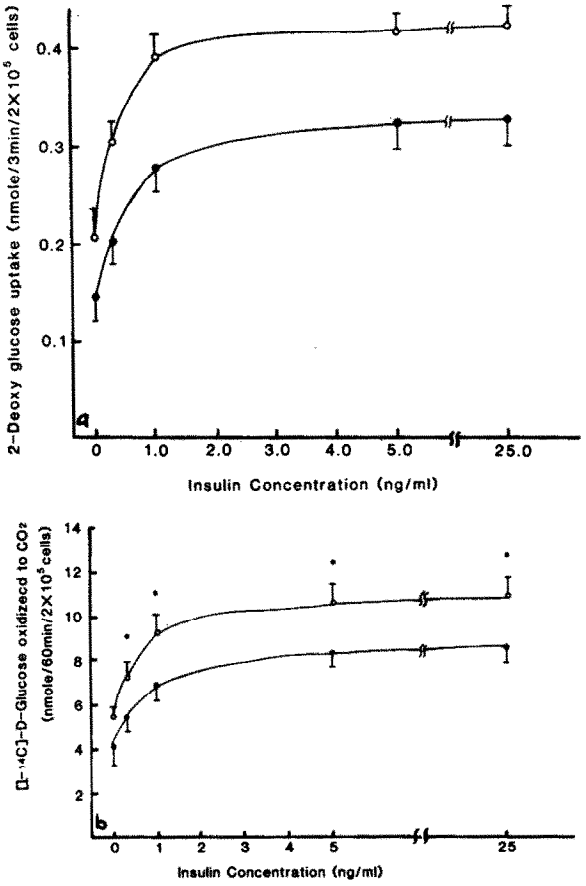


Fig.2. 2-Deoxyglucose uptake (a) and glucose oxidation (b) in adipocytes from ADD-treated (○) or non-treated (●) obese rats. Data represent the mean ± SEM of 6 separate experiments for each analysis. Differences between two groups are statistically significant at all insulin concentrations ($p < 0.05$) except for the glucose oxidation study at the basal level (without insulin).

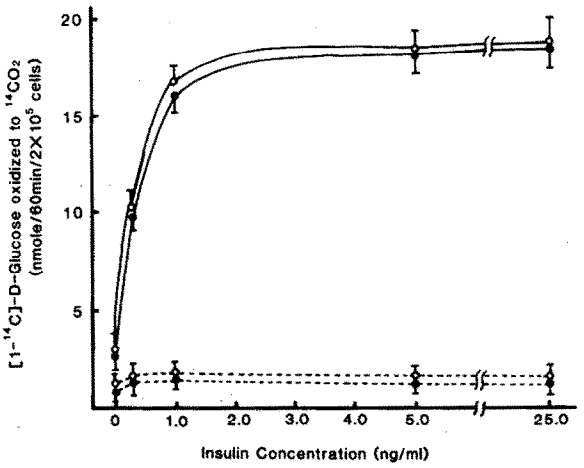


Fig.3. Glucose oxidation in adipocytes from ADD-treated (○) or non-treated (●) lean rats (——) and ADD-treated (○) or non-treated (●) streptozotocin-treated rats (---). Data represent the mean ± SEM of 4 separate experiments for each analysis. Differences between ADD-treated and non-treated groups were not statistically significant.

Thus, ADD-3878 appeared to increase the effect of circulating insulin of the rats with hyperinsulinemia and insulin resistance. Since the basal glucose uptake was elevated, the increased effect of circulating insulin may result in the increased capacity of glucose transport [11].

To further characterize the potentiating effect of ADD, adipose tissues were cultured with the agent and insulin action on these cells were determined. In the absence of insulin in the culture media, no potentiating effect of ADD on insulin action was demonstrated in the culture system (fig.4a). However, in the presence of insulin (10 ng/ml), treatment with ADD-3878 increased glucose oxidation and potentiated insulin action in vitro (fig.4b). This result agreed with the study of streptozotocin-treated rats, suggesting that the agent itself was not able to increase glucose metabolism. Insulin-binding to adipocytes after the culture with insulin, no difference was shown between the ADD-treated and non-treated groups (fig.4c). Degradation of insulin during incubation was comparable between ADD-treated and non-treated groups (43.8 ± 3.2% *vs* 41.6 ± 4.1%, mean ± SEM). Therefore, the effect of the agent appeared not to be related to a change of insulin receptors but related to the postreceptor process of insulin action.

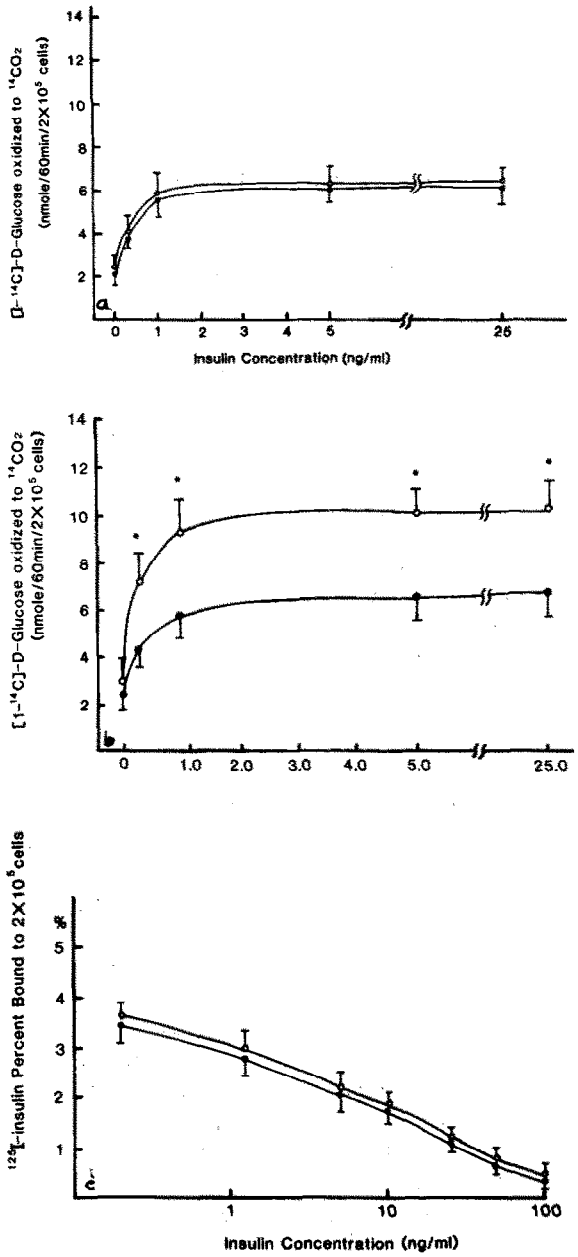


Fig.4. Effect of ADD on insulin action in vitro: (a) Glucose oxidation in adipocytes after 24 h culture with (○) and without (●) ADD in the absence of insulin; (b) Glucose oxidation in adipocytes after 24 h culture with (○) and without (●) ADD in the presence of insulin (10 ng/ml). * Differences between two groups were statistically significant ($p < 0.05$); (c) Insulin-binding to adipocytes after the culture condition of fig.4b. Differences between the two groups were not statistically significant.

The agent may act on the coupling process between receptors and effectors since no increased glucose oxidation was demonstrated at the basal level (without insulin) in the culture study, or it may be directly involved with the effector system. This agent may be useful to study the mechanism of post-receptor process of insulin action. Furthermore, because of its unique mechanism to potentiate insulin's effect in the insulin-resistant state, but not in normal or hypoinsulinemic conditions, it may be useful for treating Type-II diabetic patients, especially obese patients with hyperinsulinemia.

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