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Short communication

(*R*)-ACX is a novel sufonylurea compound with potent, quick and short-lasting hypoglycemic activity

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Abstract

We investigated the mechanism of the hypoglycemic effect of (*R*)-4-(1-acetoxyethyl)-*N*-(cyclohexylcarbamoyl)benzene-sulfonamide [(*R*)-acetoxyhexamide; (*R*)-ACX], a new sulfonylurea compound. (*R*)-ACX potently stimulated the release of insulin from cultured pancreatic β-cells (HIT T15 cells), established from hamster islet cells SV40-transformed. When (*R*)-ACX was orally administered to fasted rats, it decreased the plasma glucose level in a dose-dependent manner. The hypoglycemic effect of (*R*)-ACX was quick and short lasting, as compared to that of acetohexamide and glibenclamide. The quick and short-lasting hypoglycemic effect of (*R*)-ACX was thought likely to result from rapid absorption of (*R*)-ACX and rapid elimination of (*R*)-ACX and its metabolite, (*R*)-hydroxyhexamide. Furthermore, (*R*)-ACX was found to suppress the increase of blood glucose level due to starch loading in fasted mice. (*R*)-ACX may be useful in the control of postprandial hyperglycemia to patients with non-insulin-dependent diabetic mellitus.

Keywords: (*R*)-ACX; Sulfonylurea compound; Insulin releasing effect; Hypoglycemic effect

1. Introduction

In patients with non-insulin-dependent diabetic mellitus, the acute release of insulin after meals is known to be impaired. Thus, strict control of postprandial hyperglycemia is important in these patients to prevent chronic complications (Lebovitz, 1992; Toeller, 1992). For this purpose, a variety of sulfonylureas such as tolbutamide and glibenclamide have been used clinically. However, the prolonged hypoglycemia induced by sulfonylureas is a risk of rational therapeutics (Jackson and Bessler, 1981; Jennings et al., 1989). To control postprandial hyperglycemia and to avoid the prolonged hypoglycemia, the development of drugs having a prompt but shorter effect is expected (Ohnota et al., 1994; Ikenoue et al., 1997; Ohta et al., 1999). Previously, we reported the enzymatic synthesis of (*R*)-4-(1-acetoxyethyl)-*N*-(cyclohexylcarbamoyl)benzene-sulfonamide [(*R*)-acetoxyhexamide; (*R*)-ACX], a new sulfonylurea compound derived from acetohexamide (Akita et al., 1998). We now describe evidences that (*R*)-ACX is a promising oral antidiabetic drug with quick and short-lasting activity.

2. Materials and methods

2.1. Chemicals

(*R*)-ACX and (*R*)-hydroxyhexamide were synthesized in our laboratory as previously described (Akita et al., 1998). Acetohexamide and glibenclamide were purchased from Research Biochemicals International (Natic, MA, USA) and Sigma (St. Louis, MO, USA), respectively. All other chemicals were of reagent grade.

2.2. In vitro experiments in pancreatic β-cell (HIT T15 cell) line, established from hamster islet cells SV40-transformed

HIT T15 cells (ATCC No. CRL-1777) were purchased from Dainihon Pharmaceutical (Osaka, Japan) and cultured

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in HAM F-10 medium supplemented with 10% fetal bovine serum. The insulin-releasing effects of sulfonylurea compounds were determined according to a previously described method (Ohnota et al., 1994). Subconfluent cells in 24-well plates were washed twice with a bicarbonate buffer (pH 7.4) containing 5.55 mM glucose and 0.2% bovine serum albumin, and various concentrations of drugs were added and incubated for 30 min. Insulin in the supernatant was measured using an enzyme immunoassay kit (Glazyme Insulin EIA Test, Wako, Osaka, Japan).

2.3. In vivo experiments in fasted rats

Male Wistar rats (9 weeks old) were purchased from Charles River Japan (Atsugi, Japan) and fasted for 16 h before the experiments. Sulfonylurea compounds suspended in 0.5% sodium carboxymethylcellulose were orally administered to fasted rats. Blood samples (each 50 μl) were drawn at appropriate time intervals and centrifuged to obtain plasma. Plasma glucose levels were determined by the glucose oxidase method (Glucose-B Test, Wako). Plasma concentrations of (*R*)-ACX and its major metabolite, (*R*)-hydroxyhexamide, were determined by high-performance liquid chromatography (Takagishi et al., 1979).

2.4. In vivo experiments in fasted mice

Male ICR mice (6 weeks old) were purchased from Charles River Japan and fasted for 16 h before the experiments. Sulfonylurea compounds suspended in 0.5% sodium carboxymethylcellulose were administered orally to fasted mice 5 min before starch loading. Starch was given at a dose of 1.0 g/kg. Blood samples (each 10 μl) were drawn at appropriate time intervals and blood glucose levels were determined by the glucose oxidase method.

2.5. Statistical analysis

Statistical analysis of differences between mean values was performed using Dunnett's test. Differences with a *P*-value of less than 0.05 were considered statistically significant.

3. Results

3.1. Insulin releasing effects of (R)-ACX, (R)-hydroxyhexamide, acetohexamide and glibenclamide

Subconfluent HIT T15 cells (hamster pancreatic β-cells) were confirmed to release insulin at 5.9 ± 0.3 ng/well in 30 min. This experiment was performed in the presence of 5.55 mM glucose, according to Ohnota et al. (1994), since the insulin release did not change when the glucose concentration was raised from 5.55 to 16.5 mM. As shown in Fig. 1, insulin release was significantly stimulated by the addition of (*R*)-ACX at a concentration of 10^{-6} M. The insulin releasing effects of (*R*)-hydroxyhexamide, which is a major metabolite of (*R*)-ACX, acetohexamide and glibenclamide were also examined (Fig. 1). The insulin releasing effects of (*R*)-hydroxyhexamide and acetohexamide were smaller than that of (*R*)-ACX. In contrast, glibenclamide was found to stimulate insulin release at a concentration of 10^{-7} M.

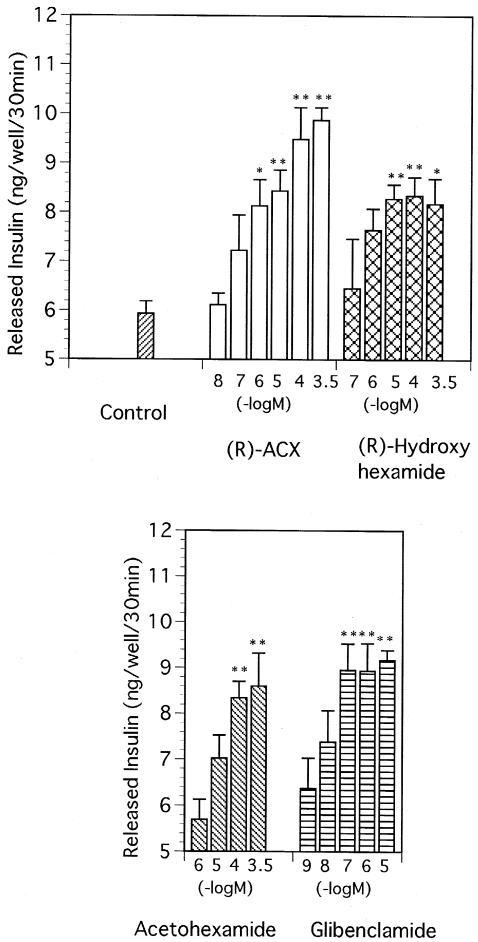

Fig. 1. Insulin releasing effects of (*R*)-ACX, (*R*)-hydroxyhexamide, acetohexamide and glibenclamide in cultured HIT T15 cells. Each bar represents the mean ± SEM for five to seven well plates. $^{*}P < 0.05$, $^{**}P < 0.01$; significantly different from the control group.

3.2. Hypoglycemic effects of (R)-ACX, acetohexamide and glibenclamide

When (*R*)-ACX, acetohexamide and glibenclamide were orally administered to fasted rats, they decreased the plasma glucose level in a dose-dependent manner (Fig. 2). The significant hypoglycemic effects of (*R*)-ACX, acetohexamide and glibenclamide were observed at doses of 3, 30, and 0.3 mg/kg, respectively. The minimum effective dose of (*R*)-ACX was about one-tenth that of acetohexamide, but about 10 times that of glibenclamide. Interestingly, the hypoglycemic effect of (*R*)-ACX was quick and short-last-

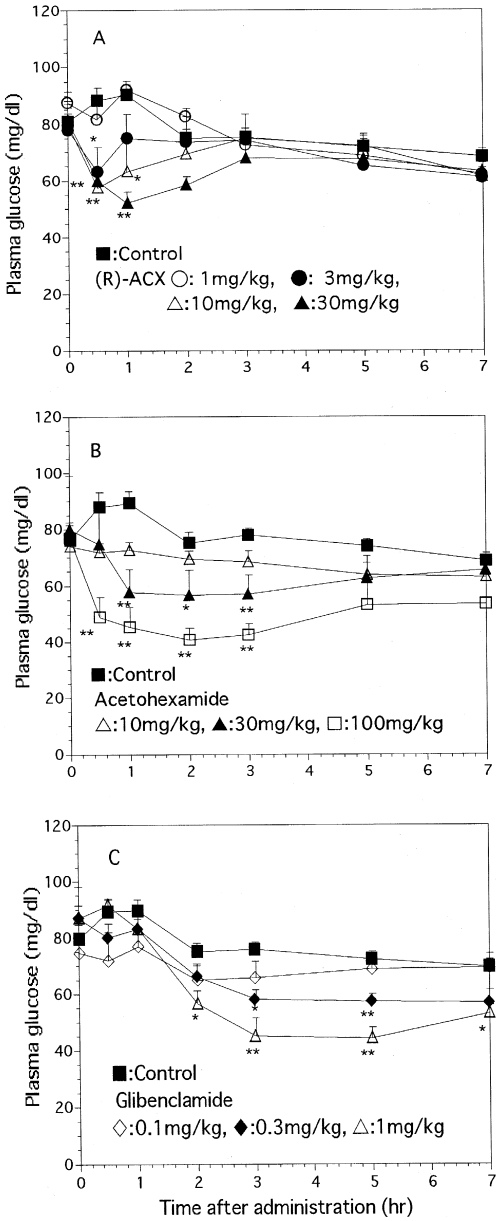

Fig. 2. Hypoglycemic effects of (*R*)-ACX (A), acetohexamide (B) and glibenclamide (C) in fasted rats. Sulfonylurea compounds and vehicle were orally administered at time zero. Each point represents the mean ± SEM for five animals. $^{*}P < 0.05$, $^{**}P < 0.01$; significantly different from the control group at the corresponding time.

ing as compared to that of acetohexamide and glibenclamide.

3.3. Plasma concentrations of (R)-ACX and its metabolite, (R)-hydroxyhexamide

(*R*)-ACX at a dose of 30 mg/kg was orally administered to fasted rats, and the plasma concentrations of (*R*)-ACX and its metabolite (*R*)-hydroxyhexamide were determined (data not shown). The plasma concentrations of (*R*)-ACX were very low, less than 1.5 μg/ml. (*R*)-Hydroxyhexamide appeared immediately in the plasma and reached its peak concentration (41.5 ± 3.7 μg/ml) at 10 min after (*R*)-ACX administration, but decreased to undetectable levels within 2.0 h.

3.4. Effects of (R)-ACX and glibenclamide on the increase in blood glucose level after starch loading

The effects of (*R*)-ACX and glibenclamide on the increase in blood glucose levels after starch loading were examined. These sulfonylurea compounds were administered orally 5 min before starch loading. (*R*)-ACX at a dose of 10 mg/kg, which has a quick and short-lasting hypoglycemic effect in fasted rats (see Fig. 2A), suppressed significantly the increase in the blood glucose level at 30 min after loading in fasted mice (data not shown). On the other hand, glibenclamide at a dose of 1 mg/kg, which induces a prolonged hypoglycemic effect in fasted rats (see Fig. 2C), did not affect the increase of the blood glucose level at 30 min after loading in fasted mice (data not shown).

4. Discussion

In the present study, we demonstrated that the oral administration of (*R*)-ACX exerts a potent, quick and short-lasting hypoglycemic effect in fasted rats. It should be noted that (*R*)-ACX is a sulfonylurea compound with quick and short-lasting hypoglycemic activity, even though the hypoglycemic potency of (*R*)-ACX was lower than that of glibenclamide. Furthermore, (*R*)-ACX was found to suppress the increase in blood glucose level due to starch loading in fasted mice, suggesting the improvement of postprandial hyperglycemia.

We found that (*R*)-ACX is rapidly hydrolyzed to (*R*)-hydroxyhexamide in fasted rats. It has been suggested that (*R*)-hydroxyhexamide, unlike (−)-hydroxyhexamide with the absolute configuration of the (*S*)-form (Akita et al., 1998), has little or no hypoglycemic activity (McMahon et al., 1965). However, in the in vitro study with cultured pancreatic β-cells, (*R*)-hydroxyhexamide was confirmed to have hypoglycemic activity. (*R*)-Hydroxyhexamide probably plays an important role in the overall hypoglycemic effect of (*R*)-ACX after oral administration.

Based on our limited data for hypoglycemic effects and plasma concentrations of (*R*)-ACX and its metabolite (*R*)-hydroxyhexamide, the quick and short-lasting hypoglycemic effect of (*R*)-ACX was likely to result from rapid absorption of (*R*)-ACX and rapid elimination of (*R*)-ACX and (*R*)-hydroxyhexamide. However, further studies are necessary to elucidate the detailed pharmacodynamics and pharmacokinetics of (*R*)-ACX and (*R*)-hydroxyhexamide.

In conclusion, the present study provided evidence that (*R*)-ACX exerts potent, quick and short-lasting hypoglycemic effects. This novel sulfonylurea compound may be useful in the control of postprandial hyperglycemia in patients with non-insulin-dependent diabetic mellitus.

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