Anti-obesity and Anti-diabetic Activities of a New $\beta_1$ Adrenergic Receptor Agonist, (S)-(Z)-[4-[[1-[2-[(2-Hydroxy-3-phenoxypropyl)]amino[ethyl]-1-propenyl]phenoxy] Acetic Acid Ethanediolic Acid (SWR-0342SA), in KK-A1 Mice

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We investigated the $\beta$-adrenergic receptor (AR) agonistic activities in rats and humans, and the anti-obesity and anti-diabetic activities in KK-A1 mice, of a new $\beta_1$-AR agonist, SWR-0342SA ((S)-(Z)-[4-[[1-[2-[(2-hydroxy-3-phenoxypropyl)]amino[ethyl]-1-propenyl]phenoxy] acetic acid ethanediolic acid). With regards to its $\beta$-AR agonistic activity in rats, SWR-0342SA stimulated the atrial beating rate ($\beta_1$-AR activity) and white adipocyte lipolysis ($\beta_2$-AR activity), but did not induce uterine muscle relaxation ($\beta_2$-AR activity). The $\beta_1$-AR agonistic activity of SWR-0342SA was about 20 times stronger than its $\beta_2$-AR agonistic activity. Similarly, SWR-0342SA enhanced the accumulation of cAMP in Chinese hamster ovary (CHO) cells expressing human $\beta_1$- and $\beta_2$-ARs, while having no effect in CHO cells expressing $\beta_2$-ARs. Adenyl cyclase stimulation by SWR-0342SA in CHO cells expressing $\beta_1$-ARs was about 35 times higher than that in CHO cells expressing $\beta_2$-ARs. With regards to anti-obesity and anti-diabetic activities, SWR-0342SA had no effect on body weight or food intake, but slightly decreased the fat pads weight in KK-A1 mice, an animal model of obesity and non-insulin-dependent diabetes mellitus (NIDDM). On the other hand, SWR-0342SA significantly decreased both blood glucose (to about 46% of control) and serum insulin levels (to about 40% of control) in KK-A1 mice.

These results indicated that SWR-0342SA is a selective $\beta_1$-AR agonist, and possesses potent anti-diabetic activity, and that the anti-obesity activity is inferior to the anti-diabetic activity.

Key words SWR-0342SA; $\beta_1$-adrenergic receptor agonist; anti-obesity; anti-diabetes

$\beta$-Adrenergic receptors ($\beta$-ARs) belong to a family of receptors that contain seven transmembrane helices, are coupled to guanine nucleotide regulatory proteins (G proteins) and are involved in the transmission of signals across the cell membrane. $\beta$-ARs mediate the physiological actions of adrenaline and noradrenaline by the activation of adenylyl cyclase via the stimulatory G-protein (Gs) which leads to an increase in theintracellular level of the second messenger, cAMP. $\beta$-ARs were initially classified into $\beta_1$- and $\beta_2$-ARs. $\beta_1$-AR is expressed mainly in the heart and $\beta_2$-AR is expressed in the uterus, skeletal muscle and in the lungs. This classification into two subtypes was later recognized to be insufficient to explain the lipolytic effects of $\beta$-AR agonists on the adipose tissue of rodents. In 1984, Arch et al. developed a novel $\beta_2$-AR agonist, BRL 37344, and showed that it was more potent at stimulating lipolysis than at increasing the heart rate or causing tracheal smooth muscle relaxation, while in contrast, isoproterenol is less potent as a stimulator of lipolysis than as a positively chronotropic and tracheal relaxant drug. This led to the hypothesis of the existence of a third, atypical, $\beta$-AR. Five years later, a cDNA clone encoding an atypical $\beta$-AR, the third $\beta$-AR, was isolated for the first time from a human genomic library, and subsequently, the existence of $\beta_3$-AR became widely accepted.

$\beta_1$-AR is expressed primarily in white (WAT) and brown (BAT) adipose tissue. Yoshida et al. showed that several $\beta_1$-AR agonists, such as BRL 26830A and CL 316,243, reduced WAT and BAT mass, and decreased the blood glucose and the serum insulin levels in obese and diabetic mice. These reports indicated that $\beta_1$-AR agonists could be useful for the treatment of obesity and/or type 2 diabetes.

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Minimization of the stimulatory effects mediated by $\beta_1$- and $\beta_2$-ARs has been a priority since they cause undesirable side effects, such as tachycardia ($\beta_1$-AR) and tremors ($\beta_2$-AR). $\beta$-AR agonists such as adrenaline, noradrenaline and isoproterenol commonly have small residues at the $\beta$-hydroxylamylaline in their structures, and are more efficient at activating $\beta_1$- and $\beta_2$-ARs than $\beta_1$-AR. For $\beta_2$-AR agonistic activity, it was reported that the presence of long and bulky residues at the $\beta$-hydroxylamylaline in their structures was essential.

In our search for selective $\beta_1$-AR agonists based on the aforementioned report, we found a novel compound, (S)-(Z)-[4-[[1-[2-[(2-hydroxy-3-phenoxypropyl)]amino[ethyl]-1-propenyl]phenoxy] acetic acid ethanediolic acid (SWR-0342SA, Fig. 1). In this study, we describe the $\beta$-AR agonistic effects of SWR-0342SA in rats and humans. Furthermore, we also examine the anti-obesity and anti-diabetic activities of SWR-0342SA in KK-A1 mice.

Fig. 1. Chemical Structure of SWR-0342SA

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MATERIALS AND METHODS

**Experimental Animals** Male and female SD rats (Japan SLC, Hamamatsu, Japan), male KK-A^N^ mice and male C57BL mice (Clea Japan, Tokyo, Japan) were used in the present study. All animals were housed in an animal room maintained at 23±2°C and 55±10% relative humidity, and had free access to food and water.

**Chemicals** (--) Isoproterenol hydrochloride, 3-isobutyl-1-methylxanthine (IBMX), estradiol benzoate, collagenase type II and bovine serum albumin (BSA) fraction V were purchased from Sigma (St. Louis, MO, U.S.A.). Ham's F-12 medium was purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). The Glucose B-Test Wako kit and the NEFA C-Test Wako kit were purchased from Wako Pure Chemicals Industries, Ltd. (Osaka, Japan). G418 was purchased from Gibco BRL (Gaithersburg, MD, U.S.A.). Fetal bovine serum was purchased from Biowhittaker (Maryland, U.S.A.). cAMP enzyme immunoassay system was purchased from Amer sham (Buckinghamshire, England). The LBIS mouse insulin enzyme immunoassay kit was purchased from Shibayagi Co., Ltd. (Gunma, Japan). SWR-0342SA was synthesized in our laboratory. All the other chemicals used were of the highest purity available.

**Cells and Media** The CHO-K1 cells, from a subclone of the parental CHO cell line, was purchased from Riken Gene Bank (Tsukuba, Japan). The cells were grown in Ham's F-12 medium containing fetal bovine serum (10% v/v), penicillin (100 U/ml) and streptomycin (100 µg/ml) at 37°C in 5% CO₂ in an air-ventilated humidified incubator. The cells were passaged using a mixture of 0.125% trypsin and 0.01% EDTA every 48 h.

**β-AR Agonistic Activity in Rats** The β-AR agonistic activity of SWR-0342SA in rats was investigated using isolated tissues or cells of the SD rat.

Stimulation of Atrial Beating Rate (β₁-AR Agonistic Activity): The rat atrial beating rate was determined in accordance with the method described by Wilson et al.¹⁰ with modification. Briefly, the whole heart was removed from male SD rats (320—360 g), and immersed in oxygenated Krebs-Henseleit solution (composition: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 7H₂O, 2.6 mM CaCl₂, 2H₂O, 24.9 mM NaHCO₃, 1.2 mM KH₂PO₄, 11.1 mM glucose, pH 7.4). The right atrial preparation was dissected away from the whole heart, and immediately mounted in a 20-ml magnus chamber filled with oxygenated Krebs-Henseleit solution. The solution was maintained at 31±1°C and continuously bubbled with 5% CO₂ in O₂. The right atrial preparation was subjected to a 0.8-g resting tension and allowed to stabilize for 60 min. Measurement of the atrial beating rate was conducted using an isometric force transducer (TB612T, Nihon Kohden, Tokyo, Japan) and a heart rate counter (AT601G, Nihon Kohden, Tokyo, Japan). After obtaining a steady baseline rate (around 200 beats/min), the preparation was exposed to cumulative concentrations of 10⁻¹⁰—10⁻⁵ M SWR-0342SA, and then to 10⁻⁶ M isoproterenol alone. The responses to SWR-0342SA were expressed as a percentage of the final response to 10⁻⁶ M isoproterenol.

**Uterine Muscle Relaxation (β₂-AR Agonistic Activity):** The rat uterine muscle relaxation response was determined in accordance with the method described by Wilson et al.¹⁰ Female SD rats (200—250 g) were induced into estrus by the injection of estradiol benzoate 1 mg/kg i.p. 24 h prior to the experiment. The two uterine horns were removed from the estrous rat. Each horn was divided longitudinally into two, thus providing four preparations from each rat. Each preparation was used for separate experiments. The preparation was placed in a 20-ml magnus chamber filled with oxygenated Krebs–Henseleit solution, which was maintained at 32±1°C. Measurement of the uterine muscle relaxation was conducted using an isometric force transducer (TB612T, Nihon Kohden, Tokyo, Japan) and a carrier amplifier (AP601G, Nihon Kohden, Tokyo, Japan). The uterine preparation was loaded to a 0.2-g resting tension and allowed to stabilize for 30 min. Then, the Na ions of the solution in the chamber were replaced by K ions (composition: 118.4 mM KCl, 1.2 mM MgSO₄, 7H₂O, 2.6 mM CaCl₂, 2H₂O, 24.9 mM KHC₂O₄, 1.2 mM KH₂PO₄, 11.1 mM glucose, pH 7.4). When steady contractions were obtained, the uterine preparation was exposed to cumulative concentrations of 10⁻¹⁰—10⁻⁵ M SWR-0342SA, and then to 10⁻⁶ M isoproterenol alone. The uterine muscle relaxation response curve to SWR-0342SA was obtained using a similar procedure to that used for determining the compound’s effects on the atrial beating rate.

**White Adipocyte Lipolysis (β₃-AR Agonistic Activity):** White adipocytes were prepared from the epididymal fat pads of male SD rats (200—250 g) according to the methods of Wilson et al.¹⁰ and Rodbell.¹¹ Briefly, fat pads were removed, chopped coarsely and digested with collagenase (6 mg/6 ml per gram of tissue) in oxygenated, modified Krebs–Henseleit solution for about 30 min in a sealed polyethylene tube placed in a shaking water bath at 37°C. The composition of modified Krebs–Henseleit solution was: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO₄, 7H₂O, 2.6 mM CaCl₂, 2H₂O, 24.9 mM NaHCO₃, 1.2 mM KH₂PO₄, 20.0 mM glucose and 2% v/v BSA, pH 7.4. The digest was centrifuged and washed with the same solution, and a white adipocyte suspension (10⁵ cells/ml) was prepared. The cell suspension was stirred to maintain homogeneity, and 450-µl aliquots were transferred to polyethylene tube. The cell suspension was exposed to 10⁻¹⁰—10⁻⁵ M SWR-0342SA or isoproterenol in final volumes of 500 µl. Incubation was carried out in the sealed polyethylene tube oxygenated with 5% CO₂ in O₂ and maintained at 37°C in a shaking water bath. The incubation was stopped after 30 min by the addition of 200 µl of 1 n H₂SO₄, and neutralized with 200 µl of 1 n NaOH. Then, the supernatants were separated by centrifugation (1500×g for 10 min at 4°C). The lipolytic response was determined by measuring the amount of non-esterified fatty acids in the aliquots of the supernatants using the NEFA C-Test Wako kit which was adjusted according to the method of Duncoumb.¹² The responses to SWR-0342SA were expressed as a percentage of the maximal response to isoproterenol.

**β-AR Agonistic Activity in Humans** β-AR agonistic activity of SWR-0342SA in humans was investigated by adenylyl cyclase stimulation in CHO cells expressing human β₁-, β₂-, or β₃-ARs.

Cloning of CHO Cells Expressing Human β-AR: Standard cloning techniques were used, as described by Maniatis et al.¹³ The human β₁-, β₂- or β₃-AR cDNA were each obtained by reverse transcription and PCR amplification from human placenta mRNA in accordance with the method de-
scribed by Granneman et al.\textsuperscript{14} with modification. Each of the PCR products was cloned into the expression vector pAP3 neo. The nucleotide sequences of each was verified with that of the human $\beta_1$-AR,\textsuperscript{15} $\beta_2$-AR\textsuperscript{16} and $\beta_3$-AR\textsuperscript{17} cDNA sequences. CHO-K1 cells were transfected with each of the pAP3 neo containing the human $\beta$-AR cDNA using the calcium-phosphate precipitation technique.\textsuperscript{18} Stable transfectants with human $\beta_1$-AR ($\beta_1$-CHO cells), $\beta_2$-AR ($\beta_2$-CHO cells) or $\beta_3$-AR ($\beta_3$-CHO cells) were obtained by culture of the cells in Ham's F-12 medium supplemented with penicillin (100 U/ml), streptomycin (100 $\mu$g/ml), fetal bovine serum (10% v/v) and G418 (1 mg/ml).

Assay of Adenylyl Cyclase Stimulation: Adenylyl cyclase stimulation was determined by the accumulation of cAMP in CHO cells according to the method of Blin et al.\textsuperscript{19} with some modification. Briefly, each of the three types of CHO cells was grown to confluence in a 96-well microplate (about $5 \times 10^5$ cells/well at confluence). After washing with Ham's F-12 medium buffered with 20 mm HEPES (pH 7.4) supplemented with 1 mm ascorbic acid and 1 mm IBMX, cell monolayers were incubated for 30 min at 37°C in 200-$\mu$l the medium containing $10^{-12}$--$10^{-5}$ M SWR-0342SA or isoproterenol. The incubation was terminated by washing once with ice-cold Ca$^{2+}$ and Mg$^{2+}$-free phosphate buffered saline (PBS(-)) and by immediate addition of 100 $\mu$l of 1 N NaOH. After a period of 20 min at 37°C, the dissolved cells were neutralized with 100 $\mu$l of 1 N acetic acid and centrifuged at 3000×$g$ for 10 min at 4°C. The concentration of cAMP in the supernatant was measured by the cAMP enzyme immunoassay system using the double antibody method. The adenylyl cyclase responses to SWR-0342SA were expressed as a percentage of the maximal response obtained with isoproterenol.

Anti-obesity and Anti-diabetic Activities in KK-A’ Mice and C57BL Mice The anti-obesity and anti-diabetic activities of SWR-0342SA were determined in accordance with the method described by Yoshida et al.\textsuperscript{20} with modification. Twelve male KK-A’ mice (7 weeks old) and 12 male C57BL mice (7 weeks old) were housed in individual cages and kept in an experimental animal room for 5 weeks. SWR-0342SA was dissolved in distilled water, and each 12-week-old animal was given orally either distilled water or SWR-0342SA at a dose of 1 mg/kg/d for 14 d. On the day following the final dose, the body weight and daily food intake of the mice were measured. After the measurements, blood was collected from the inferior vena cava, and then fat pads (epididymal, retroperitoneal and mesenteric WAT) were removed and weighed. The blood glucose level was determined with the Glucose B-Test Wako kit, which was adjusted according to the glucose oxidase method and the serum insulin level was measured with the LBIS mouse insulin enzyme immunoassay kit using the streptavidin-biotin methods.

Statistical analysis Data were expressed as mean±standard error (S.E.). The statistical differences were determined by the Student's t-test. Statistical significance was assumed when the p value was less than 0.05.

RESULTS

$\beta$-AR Agonistic Activity in Rats The dose–response curves for SWR-0342SA and isoproterenol with respect to $\beta$-

AR agonistic activity in isolated tissues or cells of the rat are shown in Fig. 2. The EC$_{50}$ values for SWR-0342SA and isoproterenol corresponding to the $\beta$-AR agonistic activity of the rat are summarized in Table 1.

Stimulation of Atrial Beating Rate ($\beta_1$-AR Agonistic Activity): With regards to atrial beating rate, both SWR-0342SA and isoproterenol showed a concentration-dependent stimulatory effect. The EC$_{50}$ values for SWR-0342SA and isoproterenol were 22.9±4.9 and 1.5±0.7 nm, respectively. The maximal response (311.3±18.5 beats/min) to SWR-0342SA was obtained at 10$^{-5}$ M, and was 85.6±3.6% of that to isoproterenol at 10$^{-6}$ M.
Table 1. Effects of SWR-0342SA and Isoprotorenon on β-AR Agnostic Activity in Rats

<table>
<thead>
<tr>
<th>Compound</th>
<th>Attral beating rat (β₂-AR)</th>
<th>Uterine muscle relaxation (β₂-AR)</th>
<th>White adipocyte lipolysis (β₂-AR)</th>
<th>Selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC_{50} (μM)</td>
<td>Max (%)</td>
<td>EC_{50} (μM)</td>
<td>Max (%)</td>
</tr>
<tr>
<td>SWR-0342SA</td>
<td>22.9±4.9</td>
<td>85.6±3.6</td>
<td>&gt;10000</td>
<td>2.9±1.3</td>
</tr>
<tr>
<td>Isoprotorenon</td>
<td>1.5±0.7</td>
<td>100</td>
<td>3.3±0.4</td>
<td>100</td>
</tr>
</tbody>
</table>

EC_{50} was determined from the concentration-response curve (Fig. 2). Maximal response of SWR-0342SA compared to that of isoprotorenon. Selectivity: ratio of EC_{50} (β₂-AR) or EC_{50} (β₂-AR) to EC_{50} (β₂-AR). Each value represents the mean±S.E. of 5 experiments.

Uterine Muscle Relaxation (β₂-AR Agnostic Activity): SWR-0342SA did not induce relaxation of K⁺-contracted rat uterine preparations at 10⁻⁵ M. On the other hand, isoprotorenon induced relaxation, and the EC_{50} value was 3.3±0.4 nM.

White Adipocyte Lipolysis (β₂-AR Agnostic Activity): In epididymal white adipocytes, SWR-0342SA and isoprotorenon enhanced lipolysis in a concentration-dependent manner. The values for SWR-0342SA and isoprotorenon were 1.2±0.1 and 17.8±4.0 nM, respectively. The maximal response (354.3±20.4 mEq/l/10^6 cells) to SWR-0342SA was obtained at 10⁻⁵ M, and was 73.0±1.8% of that to isoprotorenon at 10⁻⁵ M. The lipolytic effect of SWR-0342SA was not affected in the presence of propranolol, which is a β₁-AR and β₂-AR antagonist (data not shown).

β-AR Agnostic Activity in Humans The concentration-response curves for SWR-0342SA and isoprotorenon with respect to adenylyl cyclase stimulation (cAMP accumulation) in CHO cells expressing the three types of human β-ARs, are shown in Fig. 3. The EC_{50} values for SWR-0342SA and isoprotorenon corresponding to the cAMP accumulation are summarized in Table 2.

Adenylyl Cyclase Stimulation in β₁-CHO Cells: SWR-0342SA and isoprotorenon increased cAMP accumulation in β₁-CHO cells in a concentration-dependent manner. The EC_{50} values for SWR-0342SA and isoprotorenon were 196.1±9.9 and 0.6±0.1 nM, respectively. The maximal response (12.7±1.5 pmol/10^6 cells) to SWR-0342SA was obtained at 10⁻³ M, and was 83.8±9.8% of that to isoprotorenon at 10⁻⁵ M.

Adenylyl Cyclase Stimulation in β₂-CHO Cells: SWR-0342SA did not increase cAMP accumulation in β₂-CHO cells. On the other hand, isoprotorenon increased cAMP accumulation, and the EC_{50} value was 0.5±0.1 nM.

Adenylyl Cyclase Stimulation in β₃-CHO Cells: SWR-0342SA and isoprotorenon increased cAMP accumulation in a concentration-dependent manner in β₃-CHO cells. The EC_{50} values for SWR-0342SA and isoprotorenon were 5.4±1.2 and 5.4±0.2 nM, respectively. The maximal response (30.8±5.4 pmol/10^6 cells) to SWR-0342SA was obtained at 10⁻³ M, and was 94.2±1.4% of that to isoprotorenon.

Anti-obesity and Anti-diabetic Activities in KK-A¹ Mice and C57BL Mice The anti-obesity and anti-diabetic effects of SWR-0342SA in KK-A¹ and C57BL mice are shown in Figs. 4 and 5, respectively.

Anti-obesity Activity of SWR-0342SA: The body weight of KK-A¹ mice was greater than that of C57BL mice. SWR-0342SA did not reduce the body weight of either KK-A¹ mice or C57BL mice at a dose of 1 mg/kg/d for 14 d. The fat pads weight of KK-A¹ mice were greater than those of C57BL mice. Treatment with SWR-0342SA slightly reduced the fats pad weight in KK-A¹ mice (not significant), but not in C57BL mice. The food intake of KK-A¹ mice normally
Table 2. Effects of SWR-0342SA and Isoproterenol on cAMP Accumulation in CHO Cells Expressing the Three Types of Human β-AR

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \beta_1 )-CHO</th>
<th>( \beta_2 )-CHO</th>
<th>( \beta_3 )-CHO</th>
<th>selectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( EC_{50} ) (nm)</td>
<td>Max (%)</td>
<td>( EC_{50} ) (nm)</td>
<td>Max (%)</td>
</tr>
<tr>
<td>SWR-0342SA</td>
<td>196.1±9.5</td>
<td>83.8±9.8</td>
<td>&gt;10000</td>
<td>3.3±2.5</td>
</tr>
<tr>
<td>Isoproterenol</td>
<td>0.6±0.1</td>
<td>100</td>
<td>0.5±0.1</td>
<td>100</td>
</tr>
</tbody>
</table>

\( EC_{50} \) was determined from the concentration–response curve (Fig. 3). Max: maximal response of SWR-0342SA compared to that of isoproterenol. Selectivity: ratio of \( EC_{50} \) (\( \beta_1 \)-CHO) or \( EC_{50} \) (\( \beta_2 \)-CHO) to \( EC_{50} \) (\( \beta_3 \)-CHO). Each value represents the mean±S.E. of 3 experiments.

Fig. 4. Effects of SWR-0342SA on Body Weight, Fat Pads Weight and Food Intake in KK-A\(^{+}\) and C57BL Mice

(A) body weight, (B) fat pads weight, (C) food intake. □, distilled water; ■, SWR-0342SA. Each value represents the mean±S.E. of 5 experiments.

exceeded that of C57BL mice. SWR-0342SA had no effect on the food intake in either KK-A\(^{+}\) mice or C57BL mice.

Anti-Diabetic Activity of SWR-0342SA: The levels of blood glucose and serum insulin in KK-A\(^{+}\) mice were greater than those in C57BL mice. SWR-0342SA significantly reduced the blood glucose level (to about 46% of control: p<0.01) in KK-A\(^{+}\) mice but not in C57BL mice at a dose of 1 mg/kg/d for 14 d. Moreover, treatment with SWR-0342SA greatly decreased the serum insulin level (to about 40% of control: p<0.05) in KK-A\(^{+}\) mice but not in C57BL mice.

DISCUSSION

The present study demonstrated that SWR-0342SA, a newly synthesized phenoxy-propanol amine derivative, possessed not only \( \beta_1 \)-AR agonistic activity (i.e. lipolytic effect and adenylyl cyclase stimulation), but also anti-diabetic activity (i.e. decrease in blood glucose and serum insulin levels).

With regards to \( \beta \)-AR agonistic activity in rats, SWR-0342SA increased the atrial beating rate (\( \beta_1 \)-AR) and white adipocyte lipolysis (\( \beta_2 \)-AR), but did not induce uterine muscle relaxation (\( \beta_3 \)-AR). The selectivity of SWR-0342SA for \( \beta_1 \)-AR agonistic activity in rats, based on \( EC_{50} \) values, was approximately 20 and 8000 relative to its \( \beta_1 \)-AR and \( \beta_2 \)-AR agonistic activities, respectively (Table 1). The \( EC_{50} \) values revealed that SWR-0342SA was as potent as CL 316,243 (\( EC_{50}=3.0 \) nm) and BRL 37344 (\( EC_{50}=8.4 \) nm), which are typical \( \beta_2 \)-AR agonists in rodents. Since the lipolytic effect of SWR-0342SA was maintained in the presence of pro-
pranolol, a known β₁-AR and β₂-AR antagonist, this effect was considered as being due to β₂-AR agonistic activity (data not shown).

With regards to β-AR agonistic activity in humans, SWR-0342SA increased the accumulation of cAMP in β₁-CHO and β₂-CHO cells, but not in β₂-CHO cells, similar to the observations reported for rats. The selectivity of SWR-0342SA for adenyl cyclase stimulation in β₂-CHO cells, based on EC_{50} values, was approximately 35 and 1800 times greater than that in β₁-CHO and β₂-CHO cells, respectively (Table 2). Therefore, SWR-0342SA can probably exert its therapeutic effects in humans as well as rodents.

Because the potential order of receptor selectivity based on the EC_{50} values was β₁-AR > β₂-AR > β₂-AR in both rats and humans, this compound was suggested as being a selective β₂-AR agonist.

In terms of anti-obesity and anti-diabetic activities in KK-A^t^ mice, SWR-0342SA displayed weak anti-obesity activity as shown by the decrease of fat pads weight at a dose of 1 mg/kg/d for 14 d. The anti-obesity activity in KK-A^t^ mice was not clear compared with the lipolytic activity in white adipocytes. The cause of this phenomenon may be the low bioavailability of SWR-0342SA in KK-A^t^ mice. On the other hand, SWR-0342SA showed potent anti-diabetic activities as evidenced by the decrease of levels of blood glucose and serum insulin in KK-A^t^ mice. Although the mechanism underlying the anti-diabetic effect of SWR-0342SA has not been clarified yet, it was reported that β₂-AR agonists such as BRL 26830A and CL 316,243 increased the number of insulin receptors and restored the expression of glucose transporters (GLUT4) in the white adipose tissue of obese mice. Thus, SWR-0342SA may also improve insulin sensitivity by increasing the number of insulin receptors and/or GLUT4.

With regards to the structure of SWR-0342SA, it was shown that since the size of the amino acid residues in the groove of β₂-AR was smaller than those for β₁-AR or β₂-AR, a ligand containing long and bulky residues can probably occupy the whole space in the groove of β₂-AR. Bin et al. suggested that the efficiency of β₂-AR agonistic activity was determined by the long and bulky substituent moieties in the structure of the ligand, such as in BRL 37344, LY 79771, bucinidolol or ICI 201651. SWR-0342SA also has a long and bulky substitution at the end of the β-hydroxyamine and thus this compound may show more potent β₂-AR agonistic activity than β₁-AR and β₂-AR agonistic activities. The cause for the lack of β₂-AR agonistic activity of SWR-0342SA is not clear. It is possible that the interaction of this compound with β₂-AR is distincted from that with β₂-AR and β₂-AR. Since SWR-0342SA has the unique property of possessing β₁-AR and β₂-AR agonistic activities, but not β₂-AR agonistic activity, this compound should be of interest in the study of interactions between ligands and β-ARs.

In conclusion, the results of the present study suggest that SWR-0342SA is a selective β₂-AR agonist, and possesses potent anti-diabetic activity. On the other hand, it is also shown that the anti-obesity activity of SWR-0342SA is inferior to the anti-diabetic activity. SWR-0342SA may be a useful pharmacological tool, as well as a potential anti-diabetic agent. Further pharmacological study is necessary to determine the mechanism by which SWR-0342SA improves insulin sensitivity.

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REFERENCES