Effects of a β_3 -Adrenoceptor Agonist, BRL 26830A, on Insulin and Glucagon Release in Mice

TOSHIHIDE YOSHIDA, NORIYA HIRAOKA AND MOTOHARU KONDO

First Department of Internal Medicine, Kyoto Prefectural
University of Medicine, Kyoto 602, Japan

Abstract. The β_3 -adrenoceptor agonist, BRL 26830A, which is not inhibited by either β_1 or β_2 -selective antagonists, has been shown to possess anti-obesity and anti-diabetic actions. However, the effects of this agent on insulin and glucagon release have not yet been substantiated. Therefore, we tested the hypothesis that BRL 26830A promotes insulin and glucagon secretion via β_3 receptors on pancreatic islet B and A cells. In ICR mice fasted for 48 h, BRL 26830A significantly stimulated insulin secretion from 5 min after administration, markedly decreased blood glucose levels from 30 min after administration, and significantly increased glucagon secretion from 30 min after administration. The administration of a non-selective β -receptor antagonist, at a dose of 50 mg/kg, 30 min prior to BRL 26830A injection completely abolished the effects induced by BRL 26830A. However, the administration of a β_1 -selective antagonist at doses of 50 or 100 mg/kg did not produce any significant effects. On the action of BRL 26830A, whereas the administration of a β_2 -selective antagonist at 50 mg/kg, a near maximal effective dose, partially abolished the effects of BRL 26830A. BRL 26830A had no effect on insulin, glucagon, or glucose levels in streptozocin (STZ) diabetic mice fasted for 48 h. These results suggest that, in mice, BRL 26830A may promote insulin secretion mainly via β_3 receptors and partially via β_2 receptors on pancreatic-islet B cells, and that glucagon may be secreted as the result of hypoglycemia induced by this agent.

Key words: β_3 -Adrenoceptor agonist, Insulin, Glucagon, β_1 -Selective antagonist, β_2 -Selective antagonist.
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A HUMAN GENE that encodes a third β -adrenergic receptor, here referred to as the " β_3 -adrenergic receptor" has recently been isolated [1]. This receptor exists in brown and white adipocytes, in the liver, soleus muscle, and ileum, but not in the brain, skin, heart, or lungs [1]. These findings are based on the behavior of atypical β adrenoceptor agonists such as BRL 26830A and BRL 35135, here referred to as " β_3 -adrenoceptor agonists", that exhibit low potency at classical β_1 and β_2 receptors, but selectively

stimulate the β_3 -adrenoceptors in brown and white adipose tissues [2]. Recent studies have shown that β_3 -adrenoceptor agonists have an anti-diabetic action [3, 4] in addition to an anti-obesity action [2, 4-6]. In studies on mice and rats, it has been reported that the anti-diabetic action of these agents is due to their increasing the rate of glucose disposal, and hence glucose turnover, by stimulating insulin secretion [3, 4]. In addition, it is assumed that an increase in insulin receptors on adipocytes may contribute to the effects of these agents [7, 8]. However, at present, it is unclear whether β_3 -adrenergic receptors exist in the pancreas, and whether β_3 -adrenoceptor agonists stimulate glucagon secretion. Therefore, in order to clarify these matters, in this study we investigated the hypothesis that BRL 26830A stimulates

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Correspondence to: Dr. Toshihide YOSHIDA, First Department of Internal Medicine, Kyoto Prefectural University of Medicine, 465 Kajicho, Hirokoji, Kawaramachi, Kamikyo-ku, Kyoto 602, Japan.

insulin and glucagon secretion via β_3 -adrenergic receptors on pancreatic-islet B and A cells.

Materials and Methods

Materials

BRL 26830A, (R*, R*)-($\pm$)-methyl-4-[2-[(2-hydroxy-2-phenylethyl)amino] propyl]benzoate (Beecham Pharmaceuticals, Epsom, UK) was a gift from Dr. M. A. Cawthorne. DL-propranolol HCl (ICI, UK), metoprolol (Astra, Sweden), and ICI 118551 (ICI, UK) were gifts from the respective companies.

Animals

Two hundred and ninety-six female CRJ-ICR mice (8 weeks old, about 30g) were obtained from Charles-River Japan (Tokyo, Japan). Twenty mice fasted for 24 h were injected intraperitoneally with streptozocin (STZ) (Upjohn, Kalamazoo, MI, USA) at a dose of 200 mg/kg and 20 mice were injected with citrate buffer alone. Fourteen days after the STZ injection, 19 mice that showed hyperglycemia of > 300 mg/kg during *ad libitum* feeding were selected as STZ diabetic mice for this experiment. All these animals were allowed food and water *ad libitum*, and were kept under a controlled light-dark cycle (lights on 0600h-1800h) at $22 \pm 2^\circ\text{C}$. All animals were fasted for 48 h before experiments.

Twenty mice were given an intraperitoneal (i.p.) injection of BRL 26830A, dissolved in distilled water, at a dose of 1 or 2 mg/kg, or distilled water alone as a control. Another 20 mice were injected with a non-selective β -receptor antagonist (DL-propranolol HCl: 10, 20 or 50 mg/kg, i.p.). Another 20–33 mice were injected with a β_1 -selective antagonist (metoprolol: 50 or 100 mg/kg, i.p.) or a β_2 -selective antagonist (ICI 118551: 50, 75 or 100 mg/kg, i.p.), 30 min before the BRL 26830A injection. The 19 diabetic mice received a BRL 26830A (1 mg/kg, i.p.) injection or distilled water alone. Twenty control mice for STZ received an injection of BRL 26830A or distilled water at the same dose as the controls. Blood samples were obtained from the orbital vein plexus. Blood samples for the measurement of glucagon were immediately transferred into chilled tubes containing 1.2 mg of ethylenediamine tetraacetate (Sigma Chemical Co., USA) and 1000 KIU of aprotinin

(Trasylol, Bayer Co., Germany) per milliliter of blood [9]. These samples and other blood samples for the measurement of insulin were centrifuged immediately and the plasma and sera were stored at -20°C until used. Blood glucose was measured in whole blood by the glucose-oxidase method (FUJI DRICHEM SYSTEM, Fuji Medical System Co., Tokyo), and insulin and glucagon were measured with radioimmunoassay kits (Dainabot Co., Ltd, Tokyo).

All data are shown as the mean $\pm$ SEM. Statistical analysis was performed by analysis of variance, with significance at $p < 0.05$. Further specific group differences were determined by Duncan's multiple range test, and differences from 0 time point in individual groups were assessed by Student's *t*-test.

Results

Effect of BRL 26830A injection on blood glucose, serum insulin, and plasma glucagon levels

In mice fasted for 48 h, administration of BRL 26830A (1 mg/kg, i.p.) stimulated insulin release from a baseline level of 11.5 ± 1.1 $\mu\text{U/ml}$ before injection to 42.1 ± 4.1 $\mu\text{U/ml}$ and 21.9 ± 1.8 $\mu\text{U/ml}$, five and thirty min after injection, respectively ($P < 0.001$). Blood glucose levels were markedly decreased, when assayed 30 and 60 min after injection ($p < 0.001$). Increased plasma glucagon levels were observed 30 and 60 min after injection ($p < 0.005$). BRL 26830A at 2 mg/kg led to further increases in insulin and glucagon levels in a dose-dependent manner, and to a further greater decrease in blood glucose levels. Therefore, 1 mg/kg of BRL 26830A was selected as a suitable dose for the following experiments (Fig. 1).

Effects of the administration of a non-selective β -antagonist 30 min before BRL 26830A injection on blood glucose, serum insulin, and plasma glucagon levels

The injection of a non-selective β receptor antagonist (50 mg/kg, i.p.) 30 min prior to the injection of BRL 26830A completely prevented the effects of BRL 26830A on insulin and glucagon secretion and on the blood glucose level. However, the non-selective β antagonist, at doses of 20 and 10 mg/kg, failed to completely block these changes, although the secretion of these hormones was decreased and the reduction of

Fig. 1. Effect of BRL 26830A injection on blood glucose, serum insulin and plasma glucagon levels in mice. * $P < 0.05$, ** $P < 0.005$, *** $P < 0.001$ vs before injection, § $P < 0.05$, §§ $P < 0.01$ vs distilled water, # $P < 0.05$, ## $P < 0.01$ vs BRL 26830A (1 mg/kg).

blood glucose levels was inhibited in a dose-dependent manner at these doses (Fig. 2).

Effects of the administration of a β_1 -selective or β_2 -selective antagonist 30 min before BRL 26830A injection on blood glucose, serum insulin, and plasma glucagon levels

The injection of a β_1 -selective antagonist (metoprolol) at a dose of 50 mg/kg or 100 mg/kg 30 min before the BRL 26830A injection had no significant effect on the parameters being tested. (Data not shown since the values for 100 mg/kg

Fig. 2. Effect of a non-selective β -antagonist 30 min before BRL 26830A injection on blood glucose, serum insulin and plasma glucagon levels in mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs before injection of BRL 26830A, # $P < 0.05$, ## $P < 0.01$ vs pre-treatment of distilled water.

were very similar to those for 50 mg/kg). The administration of a β_2 -selective antagonist (ICI 118551) at a dose of 50 mg/kg 30 min before BRL 26830A injection partially prevented the effects of BRL 26830A on insulin and glucagon secretion and on blood glucose levels. However, all of the 30 mice fasted for 48 h that received the β_2 -selective antagonist at doses of 75 or 100 mg/kg died soon after the injection. Although the intraperitoneal route LD₅₀ of ICI 118551 has been reported [10] to be 72 mg/kg, 78 mg/kg, 138 mg/kg, and 128 mg/kg for male rats, female rats, male mice and

Fig. 3. Effect of the administration of β_1 -selective or β_2 -selective antagonists 30 min before BRL 26830A injection on blood glucose, serum insulin and plasma glucagon levels in mice. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs before injection of BRL 26830A, # $P < 0.05$, ## $P < 0.01$ vs pre-treatment of distilled water, § $P < 0.05$, §§ $P < 0.01$ vs DL-Propranolol.

female mice, respectively, these results suggest that 50 mg/kg is near the maximal effective dose of this antagonist (Fig. 3).

Effects of BRL 26830A injection on blood glucose, serum insulin, and plasma glucagon levels in STZ diabetic mice

In diabetic mice fasted for 48 h, 1 mg/kg of BRL 26830A had no effect on insulin, glucagon, or blood glucose levels, although in control mice BRL 26830A increased insulin and glucagon release

Fig. 4. Effect of BRL 26830A injection on blood glucose, serum insulin and plasma glucagon levels in streptozocin (STZ) diabetic and control mice. * $P < 0.05$, ** $P < 0.01$ vs before injection, # $P < 0.05$, ## $P < 0.01$ vs distilled water in control, § $P < 0.05$, §§ $P < 0.01$ vs BRL 26830A in control.

and decreased blood glucose levels. Fasting plasma glucagon levels in diabetic mice were more ($p < 0.01$) than those in controls (Fig. 4).

Discussion

In this experiment, mice fasted for 48 h were used to examine the effects of BRL 26830A on blood glucose levels, since it is known that in 24 h fasted rats BRL 26830A had no effect on blood

glucose levels in spite of its potent effects on insulin release, probably due to a release of glucose from the liver [3]. In 48-h-fasted mice, which have a very small store of liver glycogen, blood glucose levels were decreased after BRL 26830A injection, as expected.

Our present results showed that insulin release evoked by BRL 26830A was prevented by the non-selective β antagonist (DL-propranolol) at a dose of 50 mg/kg. However, a β_1 -selective antagonist (metoprolol) at doses of 50 or 100 mg/kg failed to block the insulin secretion evoked by BRL 26830A, but a β_2 -selective antagonist (ICI 118551) at a dose of 50 mg/kg, which is near the maximal effective dose, partially, but not completely, blocked the effects of BRL 26830A. As it is known that β_2 -adrenoceptor agonists promote insulin release [11, 12], our present results suggest that the insulin release evoked by BRL 26830A is promoted mainly via β_3 -adrenergic receptors and partially via β_2 -adrenergic receptors on pancreatic-islet B cells.

On the other hand, plasma glucagon levels increased after the BRL 26830A injection, following the stimulated insulin release and hypoglycemia induced by this agent (Fig. 1). BRL 26830A did not increase glucagon levels in STZ diabetic mice (Fig. 4), although fasting glucagon

levels increased in STZ diabetic mice more than in controls, a result similar to that reported previously in alloxan-treated dogs [13]. These results suggest that BRL 26830A did not promote glucagon release directly but that glucagon was secreted as a result of hypoglycemia induced by this agent.

To date, the existence of β_3 -adrenergic receptor on pancreatic-islet B and A cells has not been confirmed [1]. Therefore, pancreatic islet cell cultures are presently being used to further investigate details of insulin and glucagon release evoked by BRL 26830A.

It is concluded that, in mice, BRL 26830A may promote insulin release mainly via β_3 -receptors and partially via β_2 -receptors on pancreatic-islet B cells and that glucagon may be secreted as a result of hypoglycemia induced by this agent.

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