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RESEARCH

Synergetic Insulin Sensitizing Effect of Rimonabant and BGP-15 in Zucker-Obes Rats

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Abstract Abdominal obesity is referred for as a common pathogenic root of multiple risk factors, which include insulin resistance, dyslipidemia, hypertension, and a pro-atherogenic and pro-inflammatory state. Irrespective of its psychiatric side effects, rimonabant through blocking cannabinoid-1 receptor (CB1R) induces an increase in whole body insulin sensitivity. The aim of this work was to study the effect of selected doses of another insulin sensitizer compound BGP-15, and rimonabant on insulin resistance in Zucker obese rats with a promise of inducing insulin sensitization together at lower doses than would have been expected by rimonabant alone. We found that BGP-15 potentiates the insulin sensitizing effect of rimonabant. The combination at doses, which do not induce insulin sensitization by themselves, improved insulin signaling. Furthermore our results suggest that capsaicin-induced signal may play a role in insulin sensitizing effect of both molecules. Our data might indicate that a lower dose of rimonabant in the treatment of insulin resistance and type 2 diabetes is sufficient to administer, thus a lower incidence of the unfavorable psychiatric side effects of rimonabant are to be expected.

Keywords Rimonabant · BGP-15 · Insulin resistance · Obesity · Capsaicin · Glucose clamp

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Introduction

Overweight and obesity represent the most prevalent health problem in the developed countries. According to the estimations of World Health Organization, more than 1,4 billion adults are obese worldwide. Overweight and obesity themselves are associated with hypertension and abnormal metabolic changes such as insulin resistance and dyslipidemia which are risk factors for diabetes mellitus. Obesity (particularly abdominal obesity), insulin resistance and dyslipidemia are major features of "pre-diabetes" (metabolic syndrome) that leads to type 2 diabetes mellitus. Diabetes is accompanied by increased mortality due to a greater risk of cardiovascular diseases. Thus, it can be stated that obesity predisposes to diseases of high risk such as type 2 diabetes mellitus, cardiovascular diseases, osteoarthritis, formation of gall stones and various malignant diseases [1].

The endocannabinoid system (ECS) is an endogenous physiological system composed of two cannabinoid receptors (CB1R; CB2R) and several endogenous ligands, which are expressed widely, not only in the central nervous system but also in peripheral organs including visceral adipose tissue [2]. The ECS is intimately involved in appetite regulation energy expenditure and lipid metabolism, which makes it an intriguing target for pharmacological treatment

of obesity, diabetes, and the metabolic syndrome. In abdominal obesity, the ECS is generally up regulated in central and peripheral tissues and its blockade results in positive metabolic changes. Rimonabant (SR141716) was the first selective CB1 inverse agonist/antagonist marketed for the treatment of obesity. Large randomized trials have demonstrated efficacy in treatment of overweight and obese individuals with weight loss significantly greater than a reduced calorie diet alone. In addition, multiple other cardiometabolic parameters were improved in the treatment groups including increased levels of high density lipoprotein, cholesterol, reduced triglycerides, reduced waist circumference, improved insulin sensitivity, decreased insulin levels, and in diabetic patients improvement in glycosylated hemoglobin percentage [3]. However, psychiatric side effects, as well as depression, anxiety, irritability, and suicidal ideation may frequently occur. Therefore, it was removed from the market. The hydroximic acid derivative BGP-15 [O-(3-piperidino-2-hydroxy-1-propyl) nicotinic amidoxime dihydrocholride] is an antidiabetic drug candidate after completion of clinical phase II trial [4]. It has been shown to improve glycemic control in various in vitro and in vivo experimental models [5, 6]. We have published several data about its inducing effect on Hsp72 (heat shock protein) [7–9]. To the last of our knowledge there is no serious side effects upon BGP-15 treatment.

Capsaicin is the spicy ingredient in hot chili peppers. The capsaicin receptor, also called transient receptor potential vanilloid type-1 channel (TRPV1) is widely expressed in different tissues and organs beyond the sensory nerves and has multiple biological effects that are involved in functional regulation in the pancreas, blood vessel, adipose tissue and liver. Furthermore several findings suggest that capsaicin-sensitive nerves regulate glucose tolerance by improving insulin resistance through a mechanism that is independent of insulin release [10]. Because TRPV1+ fibers also innervate insulin target organs, including skeletal muscle and liver, TRPV1 in these organs might also be involved in glucose tolerance.

Since both rimonabant and BGP-15 induce insulin sensitization with weight loss, we postulated an at least additive or potentiating synergism between these compounds. We thought that co-administration of BGP-15 and rimonabant may render rimonabant doses significantly lower to produce sufficient metabolic effects. Experiments were undertaken to investigate the combined administration of rimonabant and BGP-15 at doses, which were ineffective on insulin resistance administered alone, in Zucker obese rats, which is an appropriate model of obesity related diseases. Furthermore we also examined the role of capsaicin pathway in the insulin sensitizing effect of rimonabant and BGP-15 on capsaicin pretreated Zucker obese rats.

Materials and Methods

Experimental Animals

The experiments were carried out with adult male Zucker obese rats weighing 400–420 g, (housed in animal room 12 h light/dark periods a day, temperature of 22–25 °C, humidity of 50–70 %) with four animals per pen. The animals were fed commercial laboratory chow and allowed free access to tap water ad libitum. The treatments began after 1-week acclimatization to the laboratory conditions.

BGP-15 was provided by N-Gene Inc.

Experimental Groups

In the 1st set of studies, the rats were divided into six groups of four animals each. Groups were treated either with saline (control group), 10 mg/kg rimonabant (ACOMPLIA Sanofi, France), 30 mg/kg rimonabant, 3 mg/kg BGP-15 b.i.d., 10 mg/kg BGP-15 b.i.d., or 3 mg/kg BGP-15 plus 10 mg/kg rimonabant twice a day for 5 days. Rats were treated orally and insulin sensitivity was determined by hyperinsulinemic euglycemic glucose clamp.

A 2nd set of experiments was carried out to study the effect of BGP-15 and rimonabant on insulin sensitivity in animals with preceding systemic capsaicin desensitization as described previously [11]. These capsaicin pre-treated animals were divided into four groups of six animals each. Groups were orally treated either with saline (positive control group), or 30 mg/kg rimonabant, 10 mg/kg BGP-15, or 30 mg/kg rimonabant plus 10 mg/kg BGP-15 for 5 days. An additional group of animals received just saline treatment (not pre-treated with capsaicin) served for negative control group.

Hyperinsulinemic Euglycemic Glucose Clamp (HEGC)

Whole body insulin sensitivity was determined by hyperinsulinemic euglycemic glucose clamping essentially as described [11]. Human regular insulin was infused at a constant rate (5–12 mU/kg/min) via a catheter inserted into one of the jugular veins over 120 min. This insulin infusion rate was adjusted to procedure 100±5 μU/ml in steady state in each species. Blood samples (0.2 ml) were taken from an arterial cannula introduced into one of the external carotid arteries for blood glucose concentration at 10-min intervals. Blood glucose concentration was maintained constant (5.5± 0.5 mmol/l) by a variable rate of glucose infusion. When blood glucose has stabilized for at least 20 min, we defined this condition as steady state. The glucose infusion rate during steady state was used to characterize insulin sensitivity.

Whole body insulin sensitivity was determined by hyperinsulinemic euglycemic glucose clamping essentially

as described [11], except clamp anesthesia. In this study, anesthesia was achieved by controlled artificial respiration with 4–10 % SEVOFLURAN subsequent to anesthesia induction by 25 mg/kg pentobarbital i.v. This was necessary, since preliminary studies revealed that insulin resistance was difficult to detect in barbiturate anesthesia.

Statistical Analysis

The results expressed as means ± S.D. were analyzed with one-way analysis of variance followed by a modified *t*-test for repeated measures according to Bonferroni's method [12]. Changes were considered significant at $P<0.05$.

Results

First Set of Experiments

In the presented 5-day long study, BGP-15 administration at a dose of 10 mg/kg but not 3 mg/kg b.i.d. significantly increased insulin sensitivity. Rimonabant at a dose of 30 mg/kg but not 10 mg/kg (oral) applied in the evening induced significant insulin sensitization as well. BGP-15 + rimonabant at doses, which do not significantly increase insulin sensitivity by themselves, induced significant insulin sensitization (Fig. 1).

Second Set of Experiments

This group of capsaicin pre-treated animals was used to study the systemic effect of capsaicin on insulin sensitivity by itself and the effects of BGP-15, rimonabant and their combination. We found that neither rimonabant nor BGP-15 increase insulin sensitivity in capsaicin-pre-treated animals, however, systemic capsaicin administration per se yields a state of increased insulin sensitivity (Fig. 2).

Discussion

The series of experiments described above demonstrate that BGP-15 potentiates the insulin sensitizing effect of rimonabant in animals with insulin resistance i.e. the rimonabant + BGP-15 combination produces a degree of insulin sensitization at much lower BGP-15 or rimonabant doses than that would have been expected at using either drug alone. It is also shown that preceding systemic capsaicin desensitization yields a state of increase in whole body insulin sensitivity that cannot be further amplified by either substance or their combination at the dose-range studied.

The results confirm our previous finding that BGP-15, a prototype of HSP co-inducer molecules increases whole body insulin sensitivity determined by hyperinsulinemic euglycemic glucose clamping in insulin resistant fasting animals [6]. Nevertheless, the model of insulin resistance used completely differs from each other in the present work and that seen in the preceding study. The Zucker obese rat, the experimental approach used in the present work is considered a suitable experimental model of human insulin resistance syndrome [13], whereas in our previous work referred, insulin resistance was attained by subchronic administration of 2nd generation antipsychotics [6]. Thus, that BGP-15 is able to counteract insulin resistance in a model of

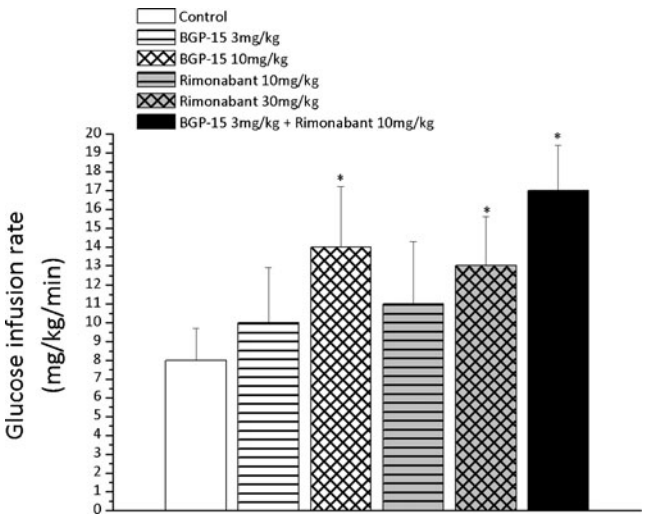

Fig. 1 The effects of BGP-15 and rimonabant on insulin sensitivity in Zucker obese male rats. The data are means ± S.D. *indicates a significant difference from control at $p<0.05$

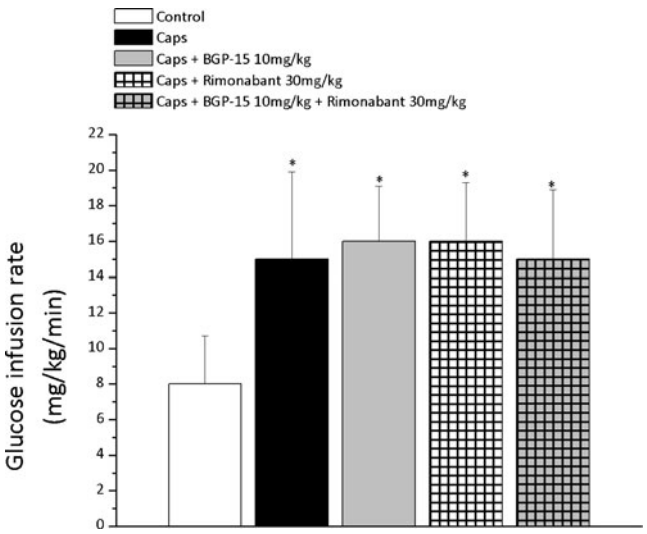

Fig. 2 The effects of BGP-15 and rimonabant on insulin resistance in Zucker obese male rats with preceding systemic capsaicin desensitization. The data are means ± S.D. *indicates a significant difference from control at $p<0.05$. *Caps* capsaicin

high relevance on human disease is one of the original observations of the present work. Similarly to BGP-15, rimonabant, a CB1 receptor antagonist, previously suggested for the treatment of obesity, also produced a dose-dependent insulin sensitization effect in Zucker rats. This drug has previously been found to decrease body weight in this model when given as long as 6 months at a dose of 10 mg/kg per day [13], the same dose as occurs in our work to exhibit a synergistic effect with BGP 15 on insulin sensitivity as an end point. Otherwise, the synergism between BGP-15 and rimonabant found in the present series of experiments is the other new finding of this work.

Although both rimonabant and BGP-15, such as their combination was able to increase insulin sensitivity in Zucker rats, these pharmacological maneuvers failed to affect whole body insulin sensitivity in Zucker rats with systemic capsaicin desensitization. However, the animals after capsaicin desensitization presented with significantly increased whole body insulin sensitivity. This latter result coincides with our observation made in healthy guinea pigs that revealed significantly increased insulin sensitivity after almost complete transient sensory neuropeptide loss due to systemic capsaicin desensitization [14]. Therefore it is suggested that insulin sensitivity once decreased, may be counteracted by either rimonabant or BGP-15, with a synergistic effect attained with their combination but a state of increased insulin sensitivity can not influenced by these compounds at least in rats.

In the past decade it is suggested that intracellular loss of heat shock proteins (HSP) play a crucial role in the development of insulin resistance and weight gain, thus type 2 diabetes [15, 16]. Weiss et al. have shown that heat shock response (HSR) sensing and signaling depends, in part on TRPV1 [17]. Activation of heat shock response improves insulin signaling, increases mitochondrial function and is associated with weight loss [8]. Kang et al. have demonstrated that capsaicin could suppress obesity-induced inflammation through nuclear factor (NF)-κB inactivation in the adipose tissues of obese mice [18].

Recent findings indicate that rimonabant inhibits the ability of lipopolysaccharide-activated macrophages to inhibit insulin signaling by reducing their production of the inflammatory cytokine tumor necrosis factor α (TNFα), thus reduces inflammation [19]. Moreover, it was demonstrated that BGP-15 administration suppresses the major mediators of insulin resistance, c-jun amino terminal kinase (JNK) and inhibitor of κB kinase (IKK) activation via increasing the expression of HSP72 [8]. Recently, we have shown that the drug candidate prolongs duration of HSF1 binding to heat shock element thus amplifies the net HSP response to stress [9].

Our results that neither rimonabant, nor BGP-15, nor their combination has additional insulin sensitizing effect on capsaicin pre-treated rats are intriguing, and may suggest that TRPV1 agonist capsaicin, rimonabant and BGP-15 increase insulin sensitivity in part in the same way by blocking inflammatory cytokines. Nevertheless further investigations are needed to evaluate the detailed role of capsaicin cascade in the mechanism of BGP-15.

Taken together, BGP-15 produces a synergistic effect with rimonabant regarding insulin sensitization. It is sufficient to administer a lower dose of rimonabant in the treatment of insulin resistance and type 2 diabetes, and a lower incidence of the unfavorable psychiatric side effects of rimonabant can be expected.

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