Beneficial Insulin-Sensitizing and Vascular Effects of S15261 in the Insulin-Resistant JCR:LA-cp Rat

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ABSTRACT

S15261, a compound developed for the oral treatment of type II diabetes, is cleaved by esterases to the fragments Y415 and S15511. The aim was to define the insulin-sensitizing effects of S15261, the cleavage products, and troglitazone and metformin in the JCR:LA-cp rat, an animal model of the obesity/insulin resistance syndrome that exhibits an associated vasculopathy and cardiovascular disease. Treatment of the animals from 8 to 12 weeks of age with S15261 or S15511 resulted in reductions in food intake and body weights, whereas Y415 had no effect. Troglitazone caused a small increase in food intake from 8 to 12 weeks of age with S15511 resulted in reductions in food intake and body weights, whereas Y415 had no effect. Troglitazone had the insulin response to the test meal, but metformin gave no improvement. S15261 decreased the expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase and stimulated the expression of acetyl-CoA carboxylase and acyl-CoA synthase. S15261 also reduced the expression of carnitine palmitoyltransferase I and hydroxymethyl-glutaryl-CoA synthase. S15261, but not troglitazone, reduced the exaggerated contractile response of mesenteric resistance vessels to norepinephrine, and increased the maximal nitric oxide-mediated relaxation. S15261, through S15511, increased insulin sensitivity, decreased insulin levels, and reduced the vasculopathy of the JCR:LA-cp rat. S15261 may thus offer effective treatment for the insulin resistance syndrome and its associated vascular complications.

Insulin resistance is an essential component of the metabolic syndrome and, together with abdominal obesity, hypertension, and hypertriglyceridemia, is a serious and growing problem that has a strong association with atherosclerosis and ischemic sequelae (Després et al., 1996). The metabolic syndrome is the early stage of a silent, and generally unrecognized, malignant process that leads to vasculopathy and atherosclerosis. This stage proceeds without any overt hyperglycemia or diabetes, yet contributes greatly to the ultimate complications, cardiovascular disease. The absence of any fully effective treatment for the insulin-resistant syndrome is a major clinical shortcoming that has prompted intensive efforts to develop new insulin-sensitizing agents and, in particular, drugs that also have cardioprotective potential. The role of insulin resistance, and the consequent hyperinsulinemia, in cardiovascular disease is only now becoming recognized. To date, treatment of patients with the metabolic syndrome has tended to focus on weight reduction through diet and, hopefully, exercise; however, the metabolic dysfunctions underlying the syndrome are complex and make such approaches both difficult and often ineffective (Després, 1998). This has led to efforts to develop pharmaceutical agents that would increase insulin sensitivity with an associated reduction in hyperinsulinemia. There has been some success in this. Drugs such as benfluorex have both lipid-lowering and insulin-sensitizing effects (Russell et al., 1997, 1998b); biguanides, such as metformin, have hypoglycemic properties and constitute an alternative approach (Inzucchi et al., 1998); and a new class of agents, the thiazolidinediones, such as troglitazone, are insulin sensitizers (Sparano and Seaton, 1998). However, none of these approaches has proven to be fully effective in treating the metabolic syndrome.

ABBREVIATIONS: cp, corpulent; NE, norepinephrine; ACh, acetylcholine; SNP, sodium nitroprusside; MTT, meal tolerance test; GK, glucokinase; Glc-6-Pase, glucose-6-phosphatase; GLUT2, glucose transporter 2; PECPK, phosphoenolpyruvate carboxykinase; FAS, fatty acid synthase; ACC, acetyl-coenzyme A carboxylase; ACS, acetyl-coenzyme A synthase; CPT, carnitine palmitoyltransferase; HMG-coenzyme A synthase, hydroxymethyl-glutaryl-coenzyme A synthase; AUC, area under the curve.
Recently, a novel oral agent, S15261, has been reported to increase insulin sensitivity in the aging Sprague-Dawley rat (Duhault et al., 1998). This compound is the L-isomer of 2-([2-methoxy-2-[3-(trifluoromethyl)phenyl]ethyl]amino)ethyl-4-([2-[[9H-9-fluoren-9-yl]acetyl]amino]ethyl)benzene and contains an ester linkage that is cleaved by plasma esterases. Cleaving yields the fragments Y415 [4-[[2-[[9H-9-fluoren-9-yl]acetyl]amino]ethyl]benzoic acid] and S15511 [(-)-5-methoxy-5-[3-(trifluoromethyl)phenyl]-3-azapentanol]. The chemical structures of S15261 and its metabolites are shown in Fig. 1. Preliminary studies have shown that S15261 enhances insulin sensitivity in some animal models (Duhault et al., 1994, 1998), suggesting that it may be an effective treatment against both the metabolic and vascular consequences of the metabolic syndrome. However, no studies have yet been conducted in an animal model showing all the elements of the metabolic syndrome, including the critically important vasculopathy.

The JCR:LA-cp rat is a unique strain that provides us with a small-animal model possessing the major elements of the metabolic syndrome, including cardiovascular disease. If homozygous for the autosomal recessive cp gene (cp/cp), the rats are obese and become insulin resistant, hyperinsulinemic, and hypertriglyceridemic (Russell et al., 1989, 1998a). If heterozygous (cp/+ or homozygous normal (+/+), the rats are lean and metabolically normal. Male cp/cp rats are atherosclerosis prone, developing advanced intimal lesions from the age of 3 months and ischemic lesions of the heart in later life (Richardson et al., 1998). The rats also exhibit a marked vasculopathy, with hyperproliferative and hyperplastic vascular smooth muscle cells (Absher et al., 1997), and both hypercontractility in response to norepinephrine (NE) and impaired nitric oxide-mediated vascular relaxation (O’Brien et al., 1998). The insulin resistance and cardiovascular dysfunctions of these animals can be modulated by several treatments (Russell et al., 1995, 1997, 1998b), suggesting that it may be possible to prevent the end-stage cardiovascular disease in both this animal model and in humans with the metabolic syndrome. We report here on treatment of the cp/cp rat with S15261 and its two metabolic products, Y415 and S15511, together with troglitazone and metformin as reference agents. Our results demonstrate that S15261 through its metabolite S15511 enabled the rats to maintain euglycemia at markedly lower plasma insulin levels. There were associated decreases in mRNA concentrations of enzymes of gluconeogenesis and increases in mRNA for enzymes of hepatic glucose utilization. Most importantly, there were improvements in the abnormal contractile and relaxation function of mesenteric resistance vessels.

Materials and Methods

Animals. Male rats of the JCR:LA-cp strain, obese (cp/cp) and lean (+/+ or a 2:1 mixture of heterozygotes (cp/+ and homozygotes (+/+)), were raised in our established breeding colony at the University of Alberta (Russell et al., 1995). The animals were weaned at 3 weeks of age and were housed, initially in pairs, in polycarbonate cages on wood chip bedding at 20°C and 55% relative humidity. Lighting was on a 12-h light/dark cycle, with either normal lighting on at 6:00 AM and off at 6:00 PM, or reversed to allow for study and testing during the dark phase of the rats' diurnal cycle, when the animals are normally active. At 6 weeks of age, the rats were housed separately and were acclimatized to the experimental room and reversed light cycle. Food was available at all times and was Teklad Rodent Diet (Harlan Sprague-Dawley Inc., Madison, WI). This is a corn- and wheat-based diet of under 4% total lipid and 23% protein content, with an energy content of approximately 3.3 kcal/g. For treatment purposes, all drugs were incorporated into powdered diet that was then moistened, pelleted by extrusion through a die, and air dried. Rats were weighed and their food consumption was measured twice a week during the treatment period. Drug concentrations in the feed were adjusted so as to maintain the desired dose of each agent on a milligram per kilogram of body weight basis. All rats were sacrificed under halothane anesthesia through bleeding from the heart. Hepatic tissue samples were taken immediately, frozen in liquid nitrogen, and maintained at -70°C. All care of the animals and experimental procedures were in conformity with the guidelines of the Canadian Council on Animal Care and were subject to institutional review and approval.

Fig. 1. The chemical structures of S15261, Y415, and S15511, shown as the free bases.
Drugs and Chemicals. S15261, S15511, and Y415 were a gift from the Institut de Recherches Internationales Servier, Courbevoie, France. Troglitazone was a generous gift from Parke-Davis (Ann Arbor, MI). Metformin, NE, acetylcholine (ACh), and sodium nitroprusside (SNP) were obtained from Sigma Chemical Co. (St. Louis, MO).

Drug Dosages. Dosages of S15261, S15511, and Y415 were chosen on the basis of previous studies in nonobese rodents and sand rats (Duhault et al., 1994; Marquèì et al., 1997). In these animals, troglitazone is effective at 50 to 200 mg/kg (Horikoshi et al., 1994). Metformin in humans has a therapeutic range of from 2 to 4 μg/mL, a plasma level that is achievable in rats with a dose of 40 mg/kg (Matthaei et al., 1993). S15261 was administered as the tartrate, to give doses of the free base of 5, 15, and 30 mg/kg of body weight on a daily basis. S15511 was given as the hydrochloride, for a dose of 15 mg/kg. Y415 was given at 15 mg/kg of the free base, and metformin and troglitazone were administered at 100 mg/kg. The stability and activity of aliquots of the drug-containing food.

Stability and troglitazone were administered at 100 mg/kg. Y415 was given at 15 mg/kg of the free base, and metformin and troglitazone were administered at 100 mg/kg. The stability and activity of aliquots of the drug-containing food. The drug was administered 2 h into the dark period, when the rats are active. The second set of rats consisted of five animals (n = 4, each group) that were treated with S15261 at 5, 15, and 30 mg/kg, or with either S15511 or Y415 at 15 mg/kg, in their feed over a 14-day period. The rats were allowed to maintain their normal activity pattern, and blood samples were taken from the tail at the beginning of the dark period (0 h) and at 12, 15, 21, and 24 h. All blood samples were immediately placed on ice, the plasma was separated, and formic acid was added to each tube to inactivate plasma esterases. Samples were frozen immediately and maintained at −70°C until assayed.

Blood Sampling and Meal Glucose Tolerance Tests. The insulin and glucose metabolism of the cp/cp rat is abnormally responsive to stress and disturbance. To reduce variability, meal tolerance tests (MTTs) were conducted on conscious rats under a specific protocol and during the dark (active) phase of their diurnal cycle (Russell et al., 1999). After being conditioned to a sham tail-bleeding procedure at the age of 7 weeks, the animals were bled from the tail at 8, 9, and 10 weeks of age at the beginning of the dark period and in the normal fed state. They were then placed on either the treated or the control diet. At 11 weeks of age, the rats were fasted over the light period and were bled from the tail at the beginning of the dark period, in the fasted state. Insulin and glucose levels were determined in the fasting state, and serum for lipid and lactate analyses was separated and frozen at −70°C until analyzed. MTTs were performed at 12 and 13 weeks of age (Russell et al., 1999).

Extraction and Northern Blot Analysis of Total RNA. For Northern blot analysis of the enzymes listed in Table 1, total RNA from frozen tissues was extracted with guanidium thiocyanate followed by purification through a CsCl cushion gradient as described by Chirgwin et al. (1979). RNA was quantified by ultraviolet absorbance at 260 nm (260/280 ratio > 1.8), and 1 μg was submitted to electrophoresis in 1% agarose gel to check the quality of the RNA preparation. Northern blot analysis of total RNA (20 μg) was performed after 1% agarose gel electrophoresis in 2.2 M formaldehyde, as previously described (Pégorier et al., 1992). Hybridization of the blots with an excess of [32P]ATP-labeled synthetic oligonucleotide specific for the 18S rRNA subunit allowed us to correct for possible variations in the amount of RNA transferred onto the membranes. The hybridization probes used were the 0.9-kb EcoRI-BamHI from pEGM4-GLUT2 (Thorens et al., 1988), the 1.8-kb fragment from pUC-OK1 (Lyndenjan et al., 1987), the 1.1-kb PstI fragment from Glc-6-Phse (Mithieux et al., 1996), the 388-bp Taq1-PstI fragment from 1IC6p-l-pyruvate kinase (Decaux et al., 1989), the 2.8-kb PstI fragment from PEPC-10 (Yoo-Warren et al., 1981), the 660-bp fragment from PpEFS (189 Neprokröeff et al., 1984), the 509-bp EcoRI fragment from p181-6ACC (Bai et al., 1986), the 530-bp EcoRV fragment from ACS cDNA (Suzuki et al., 1990), the EcoRI fragment from P61a CPT I (Esser et al., 1993) and from pBKs-CPT II 4 (Woolf et al., 1990), and the KpnI fragment from pMS1-HMG-CoA synthase (Ayté et al., 1990). Probes were radiolabeled using a multiprime DNA labeling system (Amersham, Biotech Europe, Saclay, France). Quantification was performed by scanning densitometry of the autoradiographs.

Vascular Function Studies. Studies were conducted on a subset of animals treated with 30 mg/kg S15261 and 100 mg/kg troglitazone from 8 to 12 weeks of age. All animals were studied in the nonfasted state at the end of the dark phase, as described previously (O’Brien et al., 1998). Mesenteric arcades 5 to 10 cm distal to the pylorus was removed and was placed immediately in ice-cold HEPES-buffered physiological saline (142 mmol/l NaCl, 4.7 mmol/l KCl, 1.17 mmol/l KH2PO4, 1.2 mmol/l CaCl2, 10 mmol/l HEPES, 5 mmol/l glucose). Arterial rings (approximately 300 μm in diameter) were cut to 2 mm in length and threaded onto two stainless steel wires of 25 μm in diameter and studied in an isometric myograph system (Kent Scientific Corp., Litchfield, CT). Four baths were used per experiment. Cumulative concentrations of NE (10–9–10–5 mol/l) were added to the tissue baths and the force produced was measured. Arteries were then preconstricted to 50% of their maximal constriction with NE, and cumulative concentrations of SNP, an endothelium-independent relaxing agent (10–9–10–4 mol/l), or ACh, an endothelium-dependent relaxing agent (10–9–10–6 mol/l), were added.

Analytical Methods. Plasma glucose was measured by the use of a rapid glucose oxidase technique (Beckman Instruments, Brea, CA). Insulin was assayed by a double antibody radioimmunoassay technique (Kabi Pharmacia Diagnostics AB, Uppsala, Sweden) with rat insulin standards. Serum lipid and lactate concentrations were determined as described previously (Brindley et al., 1991).

Drug Analysis. Plasma (200 μl) was analyzed for S15261 and its metabolites Y415 and S15511, using a solid phase extraction procedure by separation on Hewlett Packard series 1050 HPLC systems (Hewlett Packard, Berks, UK) with either fluorescence detection for S15261 and Y415 (Shimadzu RF 10A; Dyson Instrument Ltd., Tyne & Wear, UK) or tandem mass spectrometry (MS/MS) detection for S15511 using a VG Quattro II mass spectrometer (Micromass U.K. Ltd., Cheshire, UK). Peak identity and quantification were performed on a VG Multichrom (version 2.0; V.G. Data Systems, Cheshire, UK) for fluorescence analysis and on MassLynks (version 2.1; Micromass U.K.) for MS/MS analysis. The concentration of analyte in each sample was determined from peak height values that were referenced to calibration curves analyzed in duplicate in each analytical run. Over the calibration of 10 to 500 ng/ml, S15261 has a mean intra-assay precision of 6.0% and overall accuracy within 7% of target value. For Y415, mean precision over the analytical range of 20 to 3000 ng/ml is 2.2% and overall accuracy is within 10% of target.
S15511 was analyzed over the range of 5 to 1000 ng/ml, with a mean precision of 9.0% and mean accuracy within 6.0%.

Statistical Analyses. Data were analyzed by ANOVA and the differences were tested by Bonferroni’s method, with \( P < .05 \) taken as significant. Curve-fitting used the program SigmaPlot (SPSS Inc., Chicago, IL). Dose-response data from the vessel studies were analyzed using the program ALLFIT (De Lean et al., 1992), which fits the entire data set to the logistic equation and performs statistical comparisons between groups. Liver mRNA data were analyzed using the Wilcoxon rank-order test. All data are presented as mean ± S.E. except where otherwise noted.

Results

Pharmacokinetics. After a single dose of S15261 to naive rats, plasma concentrations of S15511 and Y415 reached peaks at 4 h of approximately 1.6 and 56 μg/ml, respectively. S15261 was not detectable in any of the plasma samples, having been converted into its two products. The plasma levels of S15511 and Y415 after treatment for 2 weeks with S15261 at three doses, and with S15511 and Y415, are shown in Fig. 2 as a function of the time of day. The data indicate that steady-state concentrations of both metabolite agents were achieved after 2 weeks of administration. The AUC\(_{24}\) for Y415 and S15511 increased proportionally with the dose given, as shown in Table 1, indicating that the pharmacokinetics of S15261 is linear within the dosing range of 5 to 30 mg/kg/day. S15511 exposure and \( C_{\text{max}} \) were similar after the administration of either S15261 at 30 mg/kg or S15511 at 15 mg/kg.

Body Weights and Food Intake. The rats were treated from 8 to 13 weeks of age. Figure 3 shows food intake and body weights for the rats treated with the different compounds, together with results from cp/cp and +/? control rats. Body weights are expressed relative to initial weights at 8 weeks of age, normalizing the initial body weight of rats in different groups and reducing the variance of results. Figure 3 shows that all doses of S15261 caused a similar, and significant, reduction (\( P < .05 \)) in food intake. Relative body weights were significantly reduced at all doses of S15261 (\( P < .0001 \)). Body weight showed a strong dose response, with the \( E_{\text{D}0} \), being approximately 5 mg/kg. Treatment with S15511 caused a significant reduction (\( P < .002 \)) in food intake and a highly significant reduction in body weight (\( P < .0001 \)). In contrast, Y415 caused no change in food intake or body weight (\( P > .05 \)). Troglitazone caused a small increase in food intake (\( P < .05 \)), whereas metformin induced no change (Fig. 3). Neither troglitazone nor metformin caused any reduction in body weight.

Insulin and Glucose Metabolism. Treatment of the rats with S15261 at 15 and 30 mg/kg over the 3-week period was associated with reduced fasting plasma insulin levels at 11 weeks of age, as shown in Fig. 4 (\( P < .05 \)). There were no significant changes in plasma glucose levels in rats treated with any of the drugs, either in the fed state at 9 and 10 weeks of age or in the fasted state at 11 weeks of age. This reflects the normoglycemic status of the cp/cp rat, which does not show plasma glucose levels any higher than its lean +/? counterpart. The effects of S15261 treatment are much more evident in the response to the test meal (Fig. 5): both cp/cp and +/? rats were able to maintain an essentially euglycemic state after the test meal, even though, in the case of the cp/cp rats, this required an extreme insulin response at 30 min. All doses of S15261 were associated with a dramatic reduction in the insulin levels of the cp/cp animals in the MTT (\( P < .002 \)), with no differences seen between the various doses of S15261. The treated rats showed no significant differences from the cp/cp control animals in plasma glucose concentra-

**Fig. 2.** Plasma concentrations of S15511 and Y415 in male cp/cp rats treated for 2 weeks with the different agents. ○, S15261, 5 mg/kg; □, S15261, 15 mg/kg; ◊, S15261, 30 mg/kg; ■, S15511 (top) and Y415 (bottom) at 15 mg/kg. Values are mean ± S.E., four rats in each group. In the group treated with S15261 at 5 mg/kg, S15511 was not measurable in the plasma. The dark period began at time 0 h.

**Fig. 3.** Food intake and relative body weights of cp/cp rats treated from 8 weeks of age. In all panels, ○, cp/cp control; ◊, +/? control. A, △, S15261, 5 mg/kg; □, S15261, 15 mg/kg; ■, S15261, 30 mg/kg. B, ◊, Y415; △, S15511. C, ◊, metformin; ◊, troglitazone. Data are mean ± S.E., eight rats in each group. Lines were fitted to the food intake data using linear regression. Relative body weight is the weight divided by the weight at 8 weeks of age, before the start of treatment, and has been fitted by a second order polynomial equation. All doses of S15261 and S15511 caused a significant reduction in food intake (\( P < .05 \)) and in the rate of increase in body weight (\( P < .0001 \)). Y415 had no significant effect on either parameter. Troglitazone and metformin had no significant effect on body weights, and troglitazone caused a small increase in food intake (\( P < .05 \)).
with a modest reduction in fed state insulin levels after 2 weeks of treatment, and in fasting plasma insulin levels after 3 weeks of treatment. Y415 caused no reduction in insulin levels. The results in Fig. 5 show that S15511 caused a very significant decrease in the insulin response to the MTT ($P < .005$), whereas Y415 induced no change.

Treatment with troglitazone for 3 weeks caused a modest decrease in plasma insulin levels that was significant only at 9 weeks of age ($P < .01$), whereas metformin had no significant effect (Fig. 4). Figure 5 shows that troglitazone significantly reduced the insulin response in the MTT ($P < .05$ at 30 min and $P < .002$ at 60 min), whereas treatment with metformin had no effect.

**Effects of S15261 on Hepatic Gene Expression.** S15261 markedly inhibited the mRNA expression for two key gluconeogenic enzymes, PEPCK and Glc-6-Pase (Table 2). In contrast, this compound stimulated the expression of genes encoding for regulatory proteins involved in glycolysis and lipogenesis (ACC and ACS) (Table 2). Consistent with this, the expression of FAS was also apparently raised, but this was not statistically significant ($P > .05$). Moreover, S15261 decreased the expression of two genes encoding for proteins that regulate the mitochondrial rate of oxidation of long-chain fatty acids (CPT I) and ketone body production (HMG-CoA synthase). Neither GLUT2, l-pyruvate kinase, ACS, nor CPT II gene expression was affected by S15261.

**Plasma Lipid and Lactate Concentrations.** There were no significant changes in the fasting plasma levels of total cholesterol (2.52 ± 0.06 mM), triglycerides (4.35 ± 0.71 mM), free fatty acids (1.29 ± 0.10 mM), or lactate (3.87 ± 0.44 mM) with the 3-week treatment with either S15261 or the related compounds. The +/− control rats, as documented in previous studies, had significantly lower concentrations of cholesterol (1.50 ± 0.07 mM, $P < .001$) and triglycerides (1.01 ± 0.30 mM, $P < .05$) than did the cp/cp rats. Although metformin did not cause any change in plasma parameters, treatment with troglitazone did result in a significant decrease in triglycerides (from 3.97 ± 0.38 to 2.33 ± 0.26 mM, $P < .005$).

**Vascular Effects.** There were significant differences between S15261-treated and control rats in the maximum constriction of mesenteric resistance vessels in response to NE

### Table 2

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Expression</th>
<th>Significance</th>
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<td>PEPCK</td>
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<td>GK</td>
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<td>FAS</td>
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<td>NS</td>
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<td>HMG-CoA synthase</td>
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l-PK, l-pyruvate kinase. NS, $P > .05$.  

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**Fig. 4.** Plasma insulin and glucose concentrations in cp/cp rats treated from 8 weeks of age, with blood samples taken in the normal fed state at 8 (before treatment), 9, and 10 weeks and in the fasted state at 11 weeks (as indicated at the top of the figure). Data are mean ± S.E., eight rats in each group. In all panels, cp/cp control; +/− control. A, △, S15261, 5 mg/kg; □, S15261, 15 mg/kg; ■, S15261, 30 mg/kg. B, ♦, Y415; ▲, S15511. C, △, metformin; □, troglitazone. None of the treated rats showed any significant differences in plasma glucose levels in any of the measurements. Treatment with S15261 at 15 and 30 mg/kg or with S15511 at 15 mg/kg resulted in lower fasting insulin levels at 11 weeks of age ($P < .05$), whereas Y415 had no effect. Troglitazone significantly reduced insulin levels, only, at 9 weeks of age ($P < .01$); metformin had no effect.

**Fig. 5.** Insulin and glucose response to a 5-g test meal by rats treated from 8 to 12 weeks of age. Data are mean ± S.E., eight rats in each group. In all panels, cp/cp control; +/− control. A, △, S15261, 5 mg/kg; □, S15261, 15 mg/kg; ■, S15261, 30 mg/kg. B, ♦, Y415; ▲, S15511. C, △, metformin; □, troglitazone. The rats, in all groups, were able to maintain euglycemia after the meal challenge, and there were no differences between groups in glucose concentrations. All S15261-treated groups showed a reduced insulin response to the test meal ($P < .002$). S15511-treated rats had lower insulin responses ($P < .005$), and the apparent decrease in Y415-treated rats was not significant ($P > .10$). Troglitazone caused a significant decrease in the insulin response at 30 and 60 min post meal ($P < .05$ and $P < .002$, respectively), whereas metformin had no effect.
Control cp/cp rats showed significantly greater maximum constriction (P < .005) than did the +/? animals. Rats treated with S15261 had a maximum contractile response significantly lower than that of the cp/cp controls (P < .05), but not different from that of the +/? animals (Fig. 6A). In contrast, troglitazone treatment did not significantly alter the vasoconstrictive response to NE (Fig. 6B). Relaxation in response to ACh was significantly impaired in the arteries from cp/cp rats compared with those from +/? animals (P < .05). Treatment with S15261, but not with troglitazone, improved maximal relaxation compared with that of arteries from +/? rats (Fig. 7). There was no significant difference in response to SNP, either in maximal relaxation or ED₅₀, in any of the groups.

Discussion

The mutation of the cp gene that results in the obesity and metabolic abnormalities of the cp/cp rat creates a stop codon in the extracellular domain of the leptin receptor (ObR) (Wu-Peng et al., 1997), leading to the absence of any membrane-bound receptors. This results in the development of a profound peripheral insulin resistance at 5 to 6 weeks of age in the cp/cp animals, with full development of the insulin resistance syndrome by 8 weeks of age (Russell et al., 1998a). Thus, the protocols used were designed for the treatment of an established condition, and not for prevention of the development of insulin resistance.

S15261 significantly reduced the food intake of the cp/cp rat at all doses studied, but not to that of the normal +/? rat. At the highest dose used, relative body weights did approach those of the +/? animals. Treatment with 15 mg/kg had effects on body weights and food intake very similar to those seen with S15261 at 30 mg/kg, which is consistent with the similar plasma levels of S15511 observed after the respective doses. Y415 was essentially without effect, confirming that S15511 is the active moiety of the parent drug in this insulin-resistant model. Loss of weight in this grossly obese rat should be considered as a beneficial effect of the treatment, especially when associated with improvements in insulin sensitivity and vascular function. However, in previous studies (Russell et al., 1997, 1998b) we have found that pair feeding and the induction of similar weight loss did not result in metabolic improvement. A drastic reduction in food intake to 60% of that of the +/? control rats is required to cause normalization of body weight and significant improvement (Russell et al., 1999).

The cp/cp rat maintains a euglycemic state, but at the expense of very high plasma insulin levels under all circumstances. Plasma insulin levels in the fasted state, or at arbitrary points of time in freely fed rats, are only weakly indicative of the true insulin/glucose metabolism. The MTT, in contrast, provides a sensitive index of the insulin response required to maintain glucose homeostasis under defined circumstances (Russell et al., 1999). The results show clearly that S15261 had a marked insulin-sensitizing effect, as evidenced by the markedly reduced insulin levels after the meal challenge (Fig. 5). This effect is due to S15511 rather than to Y415. The efficacy of S15261 and, especially, of S15511 is markedly better than that of troglitazone, which does not reduce body weight and may actually increase food intake (Fig. 1) (Horikoshi et al., 1994; Inoue et al., 1995).

Metformin has been reported to increase glucose uptake by adipocytes in fa/fa Zucker rats (Matthaei et al., 1993), although we have seen no such effect in the cp/cp rat. Matthaei et al. (1993) used a somewhat higher dose of metformin, but their results indicate that 100 mg/kg should be within the therapeutic range for rats. A major difference between the fa/fa and cp/cp rats is that the fa/fa is only leptin resistant (with some residual leptin action) and moderately insulin resistant (Pederson et al., 1991), retaining some insulin-mediated peripheral glucose uptake. The cp/cp rat, which in the absence of the ObR receptor has no known mode of leptin activity, is more hyperinsulinemic and has no insulin-mediated glucose uptake (Pederson et al., 1991; Russell et al., 1998a). This difference appears to be critical in the response to metformin. In contrast, changes in insulin and glucose metabolism after troglitazone treatment similar to those reported here have been found in fructose-fed Wistar rats (Inoue et al., 1995) and fa/fa rats (Horikoshi et al., 1994).

This work provides evidence that S15261 affects hepatic glucose and fatty acid metabolism. Indeed, the expression of genes encoding for regulatory proteins of hepatic glucose production (PEPCK and Glc-6-Pase) is reduced by S15261 treatment. In contrast, complications induced by S15261 occur despite lower insulin concentrations...
and similar blood glucose levels, suggesting that the compound has specific effects on liver metabolism.

In terms of vascular responses, the maximal constrictive response of mesenteric arteries to NE is elevated in cp/cp rats, reflecting a vascular dysfunction secondary to insulin resistance and hyperinsulinemia (O’Brien et al., 1998; Richardson et al., 1998). S15261 reduced the vasoconstrictive response to NE in the cp/cp rats, indicating a beneficial effect on vascular function. However, there was no significant effect on endothelium-independent relaxant response, suggesting a specific action on smooth muscle contractility. Endothelium-dependent relaxation to ACh is significantly impaired in the cp/cp rats, in terms of both the ED50 and maximal relaxation. S15261, but not troglitazone, significantly enhanced maximal relaxation in response to ACh, but did not reduce the EC50. We have shown that a similar improvement in vascular function is associated with a reduction in hyperinsulinemia by treatment with several agents (Russell et al., 1995, 1999b) and is accompanied by inhibition in the development of intimal lesions and ischemic myocardial damage in the cp/cp rat (Russell et al., 1995). We also have evidence that the hyper-proliferative and hyperactive character of the vascular smooth muscle cells of the cp/cp rat are prevented by intensive exercise or severe food restriction such that the development of hyperinsulinemia is essentially inhibited (Absher et al., 1999). The reduction in contractile response of the vessels to NE in the S15261-treated rats is consistent with a normalization of vascular smooth muscle cell behavior secondary to the reduction in hyperinsulinemia. Troglitazone, in contrast, had no effect on vascular response in the cp/cp rat and has also been reported to have no effect on endothelium-dependent or -independent vascular response (Tack et al., 1998). The failure to improve vascular function may be due to a limited reduction in peak insulin concentrations, as seen in the MTT. The overall results support the hypothesis that long-term S15261 treatment could have beneficial effects against the development of cardiovascular disease, and be cardioprotective, at least in the cp/cp rat.

The effective concentrations (AUC and Cmax) for the metabolite S15511 increased in proportion to the dose of S15261 given. Body weight was decreased by the treatment in a dose-dependent manner, with an ED50 of around 5 mg/kg; however, the improvement in insulin sensitivity was maximal from the lowest tested dose, suggesting a dissociation between these two effects. The decrease in weight gain could be considered as only a potential contributing factor to the improvement of the metabolic status of the corpulent rat. These findings indicate that there is no benefit in using dosages of S15261 above 5 mg/kg and that the minimal active dose of S15261 is below this value in this strain of rats. These results are consistent with previous work performed in the sand rat (Duhault et al., 1994) and the aged Sprague-Dawley rat (Marquie et al., 1997). Although the concentrations of S15511 were not measurable after the administration of 5 mg/kg of S15261, based on the linearity of the pharmacokinetics an AUC value of 0.03 μg × h/ml could be extrapolated at this dose. Considering the results obtained in healthy volunteers receiving S15261, this suggests that a daily dose of about 50 mg should be included in the first dose-ranging study in diabetic patients.

The mechanism of action of S15261 remains unknown (Duhault et al., 1998), but is very different from that of troglitazone or the biguanides, such as metformin. The primary effect is not that of reversing the insulin-resistant state directly because the reductions in fasting insulin levels are modest and do not represent a “normalization” of metabolism. Instead, the effects in the cp/cp rat are probably a better indicator of potential therapeutic efficacy than are those seen in aging, normal rats because the latter do not exhibit the full range of the insulin resistance/obesity syndrome. In contrast to the effect seen in the aging Sprague-Dawley rat, S15261 did not decrease plasma triglyceride concentrations in the cp/cp rat (Duhault et al., 1998). However, this does not preclude changes in other aspects of lipid metabolism. For instance, S15261 reduces the expression of two genes encoding for the regulatory proteins of hepatic long-chain fatty acid oxidation, CPT 1, and mitochondrial HMG-CoA synthase.

This suggests that S15261 may decrease the rate of hepatic fatty acid oxidation. Whether S15261 is also able to alter muscle fatty acid oxidation and/or i.m. triglyceride remains to be determined because these parameters are strongly associated with the development of insulin resistance (Russell et al., 1998a). The suggestion of Duhault et al. (1998) that the mechanism of action involves enhanced glucose uptake by peripheral tissues is consistent with our results. Such an effect may involve the alteration of the insulin signal transduction pathway in a long-term manner.

The present work provides evidence that S15261, through its metabolism to S15511, is effective in decreasing fasting and, especially, postprandial hyperinsulinemia in the obese insulin-resistant cp/cp rat. This effect was accompanied by a decreased contractile response of mesenteric resistance vessels to NE. The compound is presently under study in patients for the treatment of insulin resistance and holds the promise of efficacy in the prevention of the associated cardiovascular complications.

References


