REVIEW ARTICLE

Animal models of insulin resistance and cardiovascular disease: some therapeutic approaches using the JCR:LA-cp rat

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Introduction

This review will focus on work involving the use of animal models to study the efficacy and mode of action of drugs designed to treat insulin resistance and its cardiovascular complications. In particular, we will emphasize those studies that use the JCR:LA-cp rat, a strain that exhibits the principal elements of the obesity/insulin resistance syndrome, including atherosclerosis, vasculopathy, and ischaemic lesions.

Insulin Resistance and Cardiovascular Disease

The principal long-term complications of diabetes, both type 1 and type 2, are related to atherosclerosis and vascular dysfunction. In particular, atherosclerosis is strongly associated with early type 2 diabetes and a precursory insulin-resistant status. In prosperous societies, the global consequences of vascular disease, primarily heart attack and stroke, are the single greatest causes of mortality and morbidity, and evidence is growing that type 2 diabetes is a major contributor to these [1]. Until fairly recently, the most widely accepted paradigm used to explain the development of the principal vascular lesions was based on disorders of lipid metabolism and hypercholesterolaemia. This is a powerful hypothesis and accounts for the cardiovascular sequelae of genetic disorders such as familial hypercholesterolaemia. The correlation between plasma cholesterol concentrations and cardiovascular disease (CVD) in the population is not definitive, however, and cholesterol levels, on their own, are only a partial indicator of risk [2,3]. Despite the success of cholesterol-lowering therapies, the maximum primary reduction in CVD attainable in humans by this approach appears to be ≈ 35% [4,5]. Obesity and the associated metabolic syndrome that is characterized by abdominal obesity, insulin resistance and/or type 2 diabetes, and hyperlipidaemia may be the single greatest contributor to CVD through the associated metabolic dysfunctions [6–9]. However, the origins of the vascular disease in type 2 diabetes are still to be clarified, and the development of effective preventative and treatment approaches remains at the earliest stages.

Animal Models

Cardiovascular disease is a complex multifactorial phenomenon, and there are thus limited animal models for its study. The most important element of CVD is atherosclerosis and the associated vascular pathology. The classical animal model for the study of atherosclerosis has until recently been the rabbit which has been fed a high-cholesterol diet [10,11]. In a herbivore that normally does not ingest significant amounts of cholesterol in its natural habitat, such a diet does result in the development of cholesterol-laden intimal lesions. However, this model is highly abnormal and there is now evidence that the development of lesions may be related to bacterial infection, possibly through an associated chronic inflammation [12]. Further, there are no rabbit strains that spontaneously develop insulin resistance.

In recent years, several strains of mice have been proposed as models of atherosclerosis, especially the apoE knockout mouse [13]. These animals do exhibit

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intimal lesions, but essentially only after they have been induced by cholesterol feeding. The lesions are perivalvular and are not the kind of arterial atherosclerosis seen in humans [14], advanced lesions of the arterial system being absent. Attempts to make apoE knockout mice insulin resistant have been made, but without success.

On the other hand, a number of specific animal models of obesity and/or insulin resistance have become available over the past several years. These include the *ob*/*ob* and *db*/*db* mouse strains, among others, as well as rats with the two mutant genes, the *fa* and *cp*. The characteristics of these strains are summarized in table 1. In all of these mutant strains, both murine and rat, the primary defect has been found to reside in the leptin system. In particular, the *ob*/*ob* mouse produces a defective leptin protein that does not bind to the leptin receptor (or ObR) [15–17] and the *db* mouse mutation leads to a defective ObR.

In the rat strains, the *fa*, or fatty, rat gene results in an amino acid substitution in the extracellular domain of the ObR that leads to a 10-fold reduction in the binding affinity for leptin. Thus, the *fa*/*fa* rat is leptin resistant as opposed to having no functional receptor. In contrast, the *cp* rat mutation has recently been shown to create a stop codon in the extracellular domain of the ObR [18], thus possibly leading to a complete absence of the ObR in the plasma membrane of homozygous *cp*/*cp* animals. As a result of the different mutations and the different background genotypes of the rat strains, there are major metabolic and pathophysiological differences between *cp*/*cp* and *fa*/*fa* animals in the various strains incorporating these two genes [19–21]. Most critically, the JCR:LA-cp strain is unique in the spontaneous development of atherosclerosis and myocardial ischaemia–neither the *fa*/*fa* Zucker rat [20] nor the *cp*/*cp* rats of other cp rat strains develops CVD [21,22].

The JCR:LA-cp Rat

In common with the other strains incorporating the *cp* gene, the JCR:LA-cp rats, if homozygous for this gene (*cp*/*cp*), are obese, insulin resistant, and hypertriglyceridaemic [23–26]. Rats that are heterozygous (+/*cp*) or homozygous normal (+/+) are lean and not distinguishable from the parent LA/N strain. The *ob* gene, which codes for leptin itself, is of normal structure and in the *cp*/*cp* rat is identical to that of the normal Sprague Dawley rat [27]. As a result of the leptin receptor defect, circulating leptin concentrations are elevated about 30-fold in *cp*/*cp* rats (unpublished observations). The adipocyte signal to the central nervous system is thus not recognized, leading to levels of neuropeptide Y, the strongest known stimulator of feeding, being significantly higher in the arcuate nucleus of the thalamus of these animals [28,29]. As a result, the rats are highly hyperphagic.

Leptin normally decreases the secretion of insulin from the pancreas [30] and the *cp*/*cp* rat therefore exhibits an extreme hypersecretion of insulin which is much greater than in the *fa*/*fa* rat [19]. Although the deficiency of the ObR is central to the phenotype of the *cp*/*cp* rat, there is other evidence indicating that the hyperleptinaemia is not a direct cause of the vascular dysfunction, but that it is instead a precursor to, or an aggravator of [31], the insulin-resistant state which is thought to be a major determinant of the vasculopathy in these animals. The lack of response to leptin in the JCR:LA-cp rat, and possibly the diminished response in human beings, leads to the pathophysiology that is associated with the metabolic syndrome and which, in turn, gives rise to complications such as CVD.

Rats that are *cp*/*cp* are detectably obese at 3 weeks of age. A moderate hyperinsulinaemia is observed at 4 weeks, and this develops rapidly into a marked hyperinsulinaemia beyond 5 weeks of age [32]. The insulin resistance develops such that male rats, by 12 weeks of age, no longer show insulin-mediated glucose turnover [32]. The rats remain essentially normoglycaemic, but at the expense of an extreme hyperinsulinaemic response that in a standardized meal tolerance test results in plasma insulin levels of 1000 mU/l at 30 min postprandially [33]. The 30-min postprandial plasma insulin level is a highly sensitive index of insulin sensitivity and can be reduced to fasting level by some insulin-sensitizing agents [34]. The insulin-resistant state is accompanied by a decrease in the transendothelial transport of insulin, which in turn significantly lowers insulin concentrations in the peripheral tissues, especially postprandially [35]. Female *cp*/*cp* rats are less dramatically affected and show a much more modest hyperinsulinaemia and a decrease in, rather than an absence of, insulin-mediated glucose turnover and peripheral glucose uptake [23,36].

Lipid Metabolism

The profound hyperphagia and insulin resistance of the male *cp*/*cp* rats leads to the diversion of glucose derived from the carbohydrate diet into triglyceride synthesis and the secretion of very low density lipoproteins (VLDL) from the liver. Thus, the marked hypertriglyceridaemia is mainly the result of an elevated secretion, rather than a reduced clearance [23,25], of VLDL. In fact,

Table 1. Characteristics of some obese rodents

Species/strain	Mutation	Metabolic effects	Insulin status	Vascular pathology	Comments
Mouse					
ob/ob	Defective leptin	Obesity/insulin resistance		No CVD	Normalized by leptin administration
db/db	Defective ObR	Obesity/insulin resistance		No CVD	Unaffected by leptin administration
Rat					
Zucker-fa	Defective ObR	Obesity/insulin resistance; hyperlipidaemia	Moderate hyperinsulinaemia	No CVD; endothelial roughness, probably due to mycoplasma	Non-diabetic; many colonies show evidence of genetic drift
LA/N-cp	ObR truncated in the extracellular domain	Obesity/insulin resistance; hyperlipidaemia	Hyperinsulinaemia	No CVD	Non-diabetic; congenic (inbred)
SHR/N-cp	As for LA/N-cp	Obesity/insulin resistance	Hyperinsulinaemia	No artherosclerosis; perivascular fibrosis in heart	Non-diabetic; congenic; spontaneously hypertensive
SHHF/Mcc-fa^{cp}	As for LA/N-cp	Obesity/insulin resistance; hyperlipidemia	Moderate hyperinsulinaemia	Similar to SHR/N-cp	Non-diabetic; inbred; develops cardiomyopathy and congestive heart failure
ZDF/Gmi-fa	As for Zucker-fa	Obesity/insulin resistance, progressing to diabetes by 12 weeks	Early hyperinsulinaemia, dropping with age	No significant CVD	Model for fully developed type 2 diabetes
JCR:LA-cp	As for LA/N-cp	Obesity/severe insulin resistance; VLDL hyperlipidaemia	Very high insulin levels, especially postprandially	Vasculopathy; artherosclerosis; thrombosis	Non-diabetic; normotensive ischaemic lesions of the heart

CVD = cardiovascular disease; ObR = leptin receptor; VLDL = very low density lipoprotein.

in *cp*/*cp* rats lipoprotein lipase activity is increased 2.5- to fourfold in the fat depots and is 50% higher in the heart and skeletal muscle [37]. The modest increases in circulating cholesterol concentrations are due to its incorporation into VLDL and high-density lipoprotein [26]. Supplementation of the diet of the rats with as low as 0.25% cholesterol increases the total cholesterol concentration in the plasma only slightly, but produces significant increases in cholesterol ester concentrations in the VLDL fraction.

There is much evidence indicating that obese animals may show alterations in hypothalamic-pituitary-adrenal axis sensitivity or differences in responsiveness to stress compared to normal animals. Male JCR:LA-cp rats subjected to mild restraint stress for 15 min show no differences between genotypes in blood pressure, serum glucose, insulin, or corticosterone responses during or after restraint [37]. However, serum nonesterified fatty acids were shown to increase from 0.86 mM to a mean of 1.48 mM in obese rats within 10 min of restraint, and in many animals were found to be higher than 2.5 mM [37]. This response on the part of the corpulent rats indicates that the exaggerated stress response and release of fatty acids could contribute to the pathology associated with the metabolic syndrome, including the greater insulin resistance. The major cause of the marked rise in circulating fatty acids appears to be the greater mass of adipose tissue in the obese animals rather than a greater sensitivity to catecholamine-induced lipolysis. It is of note that, in human beings, android obesity is closely correlated with insulin resistance [38]. It may be that the android-obese phenotype is associated with an exaggerated release of fatty acid from intra-abdominal fat, thus producing an insulin resistance that is exhibited, to a large extent, in the liver. In the *cp*/*cp* rat, the earliest significant manifestation of the insulin resistance syndrome is the intracellular deposition of triglyceride in skeletal muscle, which occurs before the development of peripheral insulin resistance. This intracellular triglyceride accumulation is prevented by the hypolipidaemic agent, MEDICA 16, with an associated marked delay and blunting of the insulin resistance [32].

Vascular Dysfunction

In terms of the cardiovascular response, male *cp*/*cp* rats exhibit a greater noradrenergic contraction and decreased nitric oxide (NO)-mediated relaxation of the arterial vessels [39,40]. A functional defect in NO metabolism has been confirmed by the recent finding of a specific defect in NO-mediated relaxation in the coronary arteries of male *cp*/*cp* rats that is reversible with exogenous tetrahydrobiopterin [41]. This defect in NO metabolism and function may be an important contributor to vasospasm and is an important marker for endothelial dysfunction. It was recently shown that the vascular dysfunction of the *cp*/*cp* rat is associated with higher phosphodiesterase 3-A activity in the aorta [42]. This is also consistent with the increased activity and migratory characteristics of the vascular smooth muscle cells in these animals.

Increased levels of plasminogen activator inhibitor-1 (PAI-1) are associated with atherosclerosis and thrombosis [43,44]. PAI-1 is elevated in the plasma of patients with the metabolic syndrome and in atherosclerotic human aortas. There is also an increase in PAI-1 and mRNA expression in the hearts of male *cp*/*cp* rats [45]. In addition, cultured aortic rings from male *cp*/*cp* rats secrete significantly more PAI-1 than do those from either male +/+ or female *cp*/*cp* animals. These findings are consistent with observations of thrombus accumulation in the arterial system of the *cp*/*cp* rat (figure 1) [22,46–50].

Vascular Pathology

Male *cp*/*cp* rats develop atherosclerotic lesions at an early age, with raised intimal lesions being present in the aortic arch of all of these rats by 9 months of age (figure 2) [47–50]. Occlusive thrombi are often seen in both the coronary and major abdominal arteries. Accompanying the vascular lesions is an accumulation of ischaemic lesions in the myocardium that range in severity from an early necrotic stage to advanced, scarred lesions, as shown in figure 3 [22]. The intimal

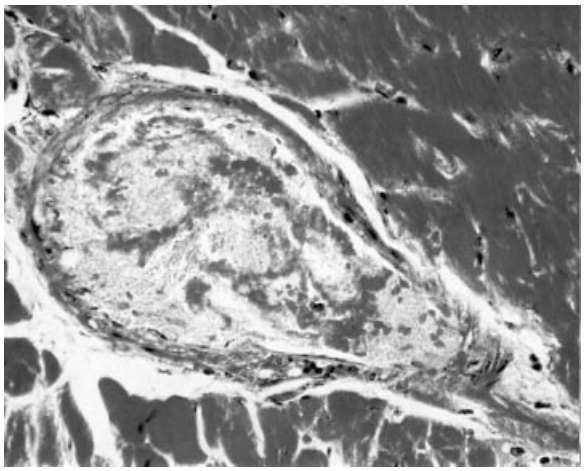

Fig. 1 Coronary artery of a 9-month-old male *cp*/*cp* rat, showing an occlusive thrombus. Haematoxylin-and-eosin staining. × 100.

lesions contain many vascular smooth muscle cells that can become lipid laden and essentially change into foam cells, as illustrated in figure 4 [49]. Consistent with the observed histological and ultrastructural changes, the smooth muscle cells of the *cp*/*cp* rat show enhanced migration from aortic explants and remain hyperproliferative in cell culture [51]. In culture, these cells show a greater proliferative response to some cytokines, including IGF-1 and FGF, as well as to insulin itself. The *in-vitro* replication rate of smooth muscle cells is a linear function of the plasma concentration of insulin in the rat from which they were derived [52]. The abnormal characteristics of these cells can be prevented or even reversed by chronic exercise or severe food restriction, both of which decrease plasma insulin concentrations [52]. These findings emphasize the central role of insulin in the pathophysiology of the JCR:LA-cp rat. The growth characteristics and response to cytokines of the vascular smooth muscle cells also provide a simple index of the vascular dysfunction and a method of quantifying the effects of experimental manipulation of these animals.

Fig. 2 Scanning electron micrograph of the lesser curve of the aortic arch of a 9-month-old male *cp*/*cp* rat. The cut edge of the vessel is evident in the foreground, with a complex raised atherosclerotic lesion on the curve below. × 200.

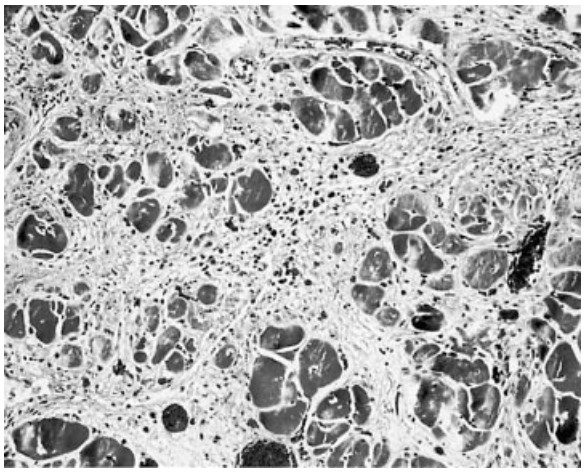

Fig. 3 Old, scarred lesion in the left ventricle of a 9-month-old male *cp*/*cp* rat, showing an area of extensive collagen or scar tissue with some remaining myocytes. Masson's trichrome staining. × 100.

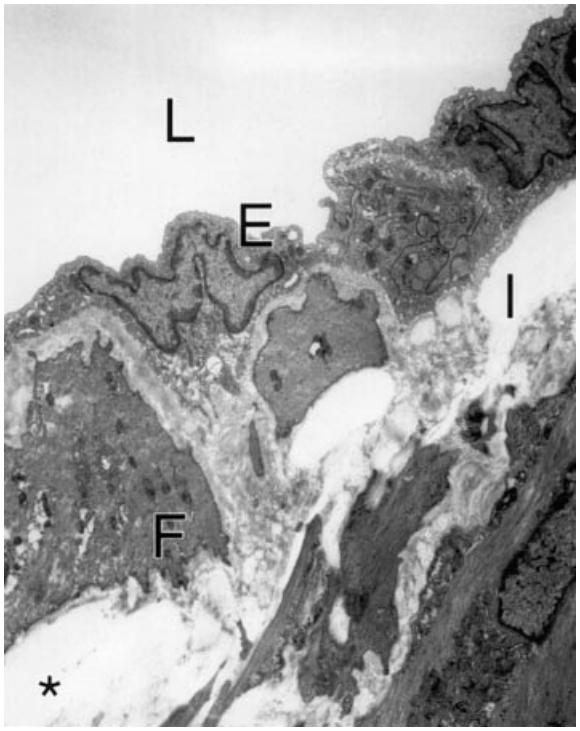

Fig. 4 Transmission electron micrograph of the aorta of a 9-month-old male *cp*/*cp* rat. L, vessel lumen; E, endothelial cell; F, smooth muscle cell or macrophage-derived cell containing lipid droplets; I, internal elastic lamina. The smooth muscle cells in the media are activated and irregular in shape. The intimal space contains activated smooth muscle cells, large lipid inclusions, and amorphous material. Micrograph courtesy of Dr Mary Richardson.

The ZDF/Gmi-fa Rat: Hyper- and Hypoinsulinaemia

The fully congenic LA/N-cp rat, while being the parent strain to the JCR:LA-cp rat, exhibits a quite different pattern of pathophysiology, being obese and insulin resistant, but having no vascular damage or dysfunction [21] (see table 1). Also in contrast to the JCR:LA-cp rat, the ZDF/Gmi-fa rat, if *fa*/*fa* and obese, moves from an insulin-resistant status to frank type 2 diabetes by 12 weeks of age [53]. The ZDF rat is not diabetic at a young age, but exhibits peripheral insulin resistance and hyperinsulinaemia by 7 weeks of age. Shortly thereafter, insulin levels of the male *fa*/*fa* rats begin to fall and these rats become diabetic, with impaired carbohydrate utilization and reduced glucose disposal [53] and plasma glucose levels above 500 mg/100 ml. In pair feeding experiments, the development of the diabetic state was shown to be prevented by caloric restriction, despite pair-fed animals having body weights identical to those of freely eating controls [54]. Similarly, both metformin and troglitazone treatment have been shown to delay the onset of diabetes and improve the insulin response [55]. Troglitazone reduced the triglyceride content of cultured pancreatic islets from ZDF rats, consistent with the lipotoxic hypothesis for adipogenic diabetes [56] and our observations of intracellular triglyceride in muscle tissue of the JCR:LA-cp rat [32]. There is also evidence for a direct role of leptin in the development of the insulin resistance and diabetes in the *fa*/*fa* ZDF rat [57,58]. This rat differs from the *cp*/*cp* rat in its retention of some limited leptin binding capacity at the receptor. The failure of the *cp*/*cp* rat of the JCR:LA-cp strain to respond to food restriction and troglitazone treatment in the same way as the *fa*/*fa* ZDF rat is consistent with the different defects in the ObR induced by the mutations. It would appear that the *cp*/*cp* rat is more severely affected, having a more profound hyperinsulinaemia; yet, unlike the ZDF rat, it does not lose pancreatic function and become diabetic. This difference must reflect differences in other, as-yet unknown elements of the genome. The marked vascular damage seen in the JCR:LA-cp strain is, in our opinion, probably due to the extreme hyperinsulinaemia [33]. Interestingly, both the ZDF and JCR:LA-cp are sensitive to cholesterol feeding, which is manifested as exacerbated myocardial injury in the ZDF rat [59] and as enhanced atherosclerosis in the JCR:LA-cp (unpublished observations).

These rat strains provide complementary models for the study of the two important aspects of the obesity/insulin resistance syndrome seen in humans. The JCR:LA-cp strain shows the early hyperinsulinaemic and silent phase during which the insidious process of atherogenesis and vascular damage occurs, thus allowing for the study of the mechanisms that lead to advanced pathological lesions and research into putative treatment agents. The ZDF rat, on the other hand, with its early development of insulin secretion failure, allows for the study of the progression to overt diabetes, the effects of the diabetic hyperglycaemic state, and the prevention and treatment of this major clinical problem in humans.

In summary, when homozygous cp/cp, the JCR:LA-cp rat exhibits the classical features of insulin resistance, hyperinsulinaemia, hypertriglyceridaemia, and obesity that are associated with the metabolic syndrome, along with the unique dimension of the end-stage pathological sequelae. It must be emphasized that these rats are not diabetic, but rather exhibit euglycaemia and thus resemble humans in the early, silent stages of the insulin–resistant syndrome long before its progression to overt type 2 diabetes. Thus, the JCR:LA-cp rat provides a very good model for screening and investigating the mode of action of insulin-sensitizing, antiatherosclerotic, and cardioprotective drugs, whereas the ZDF/GMI-fa rat provides a complementary model for similar studies in a model of the late stage disease with overt type 2 diabetes.

Some Clinical Implications

Type 2 diabetes is a difficult clinical problem, mainly because the diabetes is a symptom of a complex and insidious disease process that primarily leads to macrovascular end-stage disease. The diabetic state only becomes manifest at a late stage when the hyperinsulinaemic state slides into insulin deficiency (relative or absolute). Our studies on the insulin-resistant and hyperinsulinaemic *cp*/*cp* rat make evident a relationship between high plasma insulin levels, either basal or, possibly more serious, episodic postprandial surges, and vascular disease. The data suggest that the most serious effects are on the medial vascular smooth muscle cells, that show permanently altered growth and functional characteristics. From a clinical perspective, this suggests that important macrovascular sequelae of type 2 diabetes are initiated by events during the silent, early stages. Prevention of the end-stage cardiovascular disease, then, must depend upon early diagnosis at the non-diabetic, hyperinsulinaemic phase. Such diagnosis is possible, but will require the development of a new metabolic test regimen that addresses the basal and postprandial insulin response of patients.

A possible clinical approach would be to perform such a test on individuals at high risk of later type 2 diabetes, especially those with significant abdominal obesity and/or hypertriglyceridaemia.

Research with Novel Insulin-Sensitizing Agents

D-fenfluramine

Treatment of *cp*/*cp* rats of the JCR:LA-cp strain with D-fenfluramine, an anorectic agent, has been shown to improve insulin sensitivity and reduce plasma insulin levels [60,61]. D-fenfluramine also markedly increases the relaxant response of arterial rings to acetylcholine, indicating an improvement in the defective NO-mediated relaxation system [60]. The severity of the lesions of the aortic arch and the frequency of myocardial lesions are also markedly reduced [61]. These beneficial effects of D-fenfluramine treatment are not evident in matched food-restricted control animals, indicating direct pharmacological effects unrelated to the metabolic effects of reduced body weight.

PPAR Agonists

Binding to the PPAR receptor sites has effects on many intracellular functions, including insulin metabolism. Fenofibrate, primarily a PPAR-α agonist, has been shown to improve insulin sensitivity of the JCR:LA-cp rat without reducing the VLDL hyperlipidaemia. Troglitazone, a PPAR-γ agonist, also reduces insulin levels, but has no effect on plasma lipid concentrations or on vascular hypercontractility [34]. The hypolipidaemic agent MEDICA 16 is also a PPAR-α agonist (with additional effects on other systems), and some of its insulin-sensitizing effects and the improvement in vascular function in the *cp*/*cp* rat with treatment may be due to its effects on the PPAR system. When administered to JCR:LA-cp rats from 6 weeks of age, when the insulin resistance syndrome has just become established, it causes a significant reduction in plasma insulin concentrations. There is also an accompanying improvement in NO-mediated vascular relaxation, a reduction in the severity of aortic lesions, and an essential prevention of myocardial lesions [62].

These preliminary results indicate that the PPAR system plays a role in the metabolic dysfunction of the insulin-resistant *cp*/*cp* rat, but do not as yet allow for any conclusions regarding the mechanisms involved. However, these and other studies have established that the CVD of the obesity/insulin resistance syndrome of the *cp*/*cp* rat is treatable and potentially preventable.

S15261

The novel compound S15261, 2-([2-methoxy-2-[3-(trifluoromethyl)phenyl]ethyl}amino)ethyl-4-(2-{[2-(9H-9-fluorylenyl)acetyl]amino}ethyl)benzene, has been developed for the oral treatment of insulin resistance and type 2 diabetes [63]. This compound is cleaved in the plasma by esterases to the fragments Y415 and S15511. Studies in which the *cp*/*cp* rats were treated from 8 to 12 weeks of age with either S15261 or S15511 showed a significant decrease in their food intake and body weights, whereas treatment with Y415 had no significant effects on these parameters [34]. In contrast, troglitazone, used as a reference compound in these studies, caused a small increase in food intake, and metformin, a further reference compound, had no significant effect. Treatment with S15261 or S15511 decreased postabsorptive plasma insulin levels. Y415 had no significant effect, in contrast to its enhancement of glucose uptake in normal rats [64] and sand rats [65]. Meal tolerance tests revealed that treatment with either S15261 or S15511 essentially prevented the marked postprandial peak in insulin concentrations seen in male *cp*/*cp* rats, whereas treatment with Y415 had no significant effect. Troglitazone, in contrast, halved the insulin response to the test meal, whereas metformin had no significant effect. Thus, S15261 and S15511 increase insulin sensitivity and enable the rats to maintain euglycaemia without the marked postprandial peak normally seen in insulin concentrations. In terms of cardiovascular responses, S15261, but not troglitazone, decreased the exaggerated contractile responses of mesenteric resistant vessels to noradrenaline and increased maximum NO-mediated relaxation in arterial rings.

Conclusion

Cardiovascular disease remains the critical and, to date, quite intractable complication of diabetes. With the increasing incidence of obesity and the associated insulin resistance, hyperinsulinaemia, and type 2 diabetes in the human population, the related CVD has become a major public health problem. The development of effective preventative and treatment regimens will depend greatly on the use of appropriate animal models. The various rat strains now available for research give us the ability to conduct the necessary physiological and pharmacological studies. The results

obtained to date with a very limited range of compounds are highly encouraging and indicate that an effective treatment for established insulin resistance is possible through more than one route. Success in this endeavour would open the prospect of reducing the growing disease burden incurred by the metabolic syndrome in the human population.

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