Contrasting Metabolic Effects of Antihypertensive Agents

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Received May 9, 2003; accepted August 21, 2003

ABSTRACT
Hypertension often coexists with hyperlipidemia, insulin resistance, and glucose intolerance, a comorbidity known as metabolic syndrome X. Different antihypertensives have mixed effects on these associated abnormalities. We compared three antihypertensives in the spontaneously hypertensive obese rat model of syndrome X. Moxonidine (4 mg/kg), an imidazoline and $\alpha_2$-adrenergic agonist, $\alpha$-methyldopa (200 mg/kg), an $\alpha_2$-adrenergic agonist, or the vasodilator hydralazine (10 mg/kg) was given orally for 15 d. All three agents lowered blood pressure equally. Moxonidine significantly reduced fasting plasma insulin, glucagon, cholesterol, triglycerides, and free fatty acids (FFA) compared with untreated controls. In contrast, syndrome X markers were not affected by $\alpha$-methyldopa treatment, and hydralazine reduced only glucagon and FFA. Relative to untreated controls, moxonidine improved glucose tolerance as shown by reduced glucose area under the curve (AUC) (13.6 ± 2.4 versus 42.5 ± 9.9 g · min/dl). Insulin AUC was increased (7.4 ± 0.9 versus 3.9 ± 1.8 $\mu$g · min/ml) as was the plasma C-peptide response to the glucose load. In contrast, $\alpha$-methyldopa and hydralazine worsened glucose tolerance (68 ± 26 and 110 ± 21 g · min/ml, respectively) and significantly reduced insulin AUC (2.5 ± 0.8 and 2.3 ± 1.0 $\mu$g · min/ml, respectively) compared with controls. Moxonidine reduced but $\alpha$-methyl-

dopa and hydralazine elevated glucagon levels after the glucose load. Contrary to the “hemodynamic hypothesis” for the metabolic actions of antihypertensives, which predicts roughly equal benefits, only moxonidine had a positive impact on comorbidities. This unique action suggests a role for direct stimulation of imidazoline receptors.

Many therapeutic agents have established efficacy in hypertension. However, hypertension rarely occurs in isolation. Metabolic syndrome X is a cluster of metabolic diseases, including hypertension, insulin resistance, hyperlipidemia, glucose intolerance, and obesity. This syndrome frequently precedes the development of type II diabetes and atherosclerosis. The obese spontaneously hypertensive rat (SHROB; Koletsy sky rat) is a unique animal model of metabolic syndrome X with genetic obesity superimposed on a background of genetic hypertension (Koletsky et al., 2001). The obese phenotype results from a nonsense mutation in the leptin receptor gene, designated $f_{a^k}$, which is a naturally occurring knockout of all forms of the leptin receptor (Takaya et al., 1996). The $f_{a^k}$ mutation imposed on a hypertensive background results in extreme hyperinsulinemia, hyperlipidemia, glucose intolerance, and decreased expression of insulin signaling proteins in skeletal muscle and liver (Friedman et al., 1997).

Antihypertensive agents differ in their impact on glucose and lipid homeostasis. Human studies are not unanimous, but in general thiazide diuretics and $\beta$-adrenergic antagonists have slight adverse effects, calcium channel blockers are mixed, and $\alpha_1$-antagonists and inhibitors of the renin-angiotensin system have positive effects (Rabbia et al., 2001; Imazu, 2002). Several theories have been advanced to account for the metabolic effects of various classes of antihypertensives. A prevalent theory that could be called the “hemodynamic hypothesis” postulates that substrate and hormone delivery to target tissues is a major limitation on glucose disposal in hypertension (Julius et al., 1992). Thus, antihypertensives with direct or indirect vasodilating actions will improve glucose disposal, whereas agents such as $\beta$-blockers that reduce cardiac output and increase vascular resistance will worsen glucose disposal. The supporting evidence for this hypothesis comes from clinical drug trials and tests of human forearm microcirculation but few laboratory trials have been carried out.

A related hypothesis postulates a central role for the sympathoadrenal system in the integration of cardiovascular and metabolic actions of drugs (Julius et al., 1992; Ernsberger et

ABBREVIATIONS: SHROB, spontaneously hypertensive rat(s), obese substrain; FFA, plasma free fatty acids; l,R, l,-imidazoline receptor; $\alpha_2$AR, $\alpha_2$-adrenergic receptor; SHR, spontaneously hypertensive rat; OGGT, oral glucose tolerance test; AUC, area under the curve; ANOVA, analysis of variance.

This study was supported by HL44514 from the National Institutes of Health and by a grant from Solvay Pharmaceuticals (Hannover, Germany). This work was submitted in partial fulfillment of the requirements for a doctorate in Nutrition from Case Western Reserve University School of Medicine.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

DOI: 10.1124/jpet.103.054221.
In addition to possible hemodynamic effects, sympathoadrenal activation inhibits insulin secretion and promotes glucagon secretion from the pancreas, activates glycogenolysis and gluconeogenesis and elevates FFA among other possibly adverse effects (Ernsberger et al., 1998). Thus, sympatholytic agents such as α1-antagonists and centrally acting antihypertensives may have beneficial metabolic effects. Conversely, antihypertensive agents that evoke reflex sympathoexcitation may have adverse metabolic effects (Jamerson et al., 1993). The beneficial effects of α1-adrenergic antagonists on both glucose and lipid metabolism have been extensively studied in humans and animals, and several recent studies indicate that central sympatholytic agents of the imidazoline class improve glucose metabolism in humans and in animal models (Ernsberger et al., 1996, 1999; Henrik sen et al., 1997; Rosen et al., 1997; Haenni and Lithell, 1999; Yakubu-Madus et al., 1999; Bauduceau et al., 2000; De Luca et al., 2000; Esler et al., 2001).

The prototypical imidazoline central sympatholytic agents are moxonidine and rilminaline (Chan and Head, 1996). They are agonists at the I1 imidazoline receptor (I1R) as well as α2AR and act in the medulla oblongata to inhibit the sympathoadrenal system and lower blood pressure. Moxonidine's affinity at I1R affinity is 40 times greater than at α1R (Ernsberger et al., 1993), but a contribution of α2AR to its actions cannot be ruled out (Szabo et al., 2001). Clinical studies suggest that treatment of hypertension with the antihypertensive agent moxonidine may lower glucose levels in hyperglycemic patients (Haenni and Lithell, 1999). Studies in several experimental models, including spontaneous hypertensive obese rats and lean spontaneous hypertensive rats (SHR) (Ernsberger et al., 1996, 1999) and in fructose-fed hypertensive rats (Rosen et al., 1997) all showed that chronic moxonidine therapy improves glucose tolerance and the insulin response to a glucose load. Indeed, chronic moxonidine therapy has been shown to enhance skeletal muscle glucose transport in insulin-resistant obese Zucker rats in vitro (Henriksen et al., 1997). Chronic moxonidine treatment also improved glucose homeostasis in Zucker diabetic fatty rats (Yakubu-Madus et al., 1999). None of these studies examined the question of which receptor(s), I1R, α2AR, or both, mediate these metabolic effects, but it has been hypothesized that I1R located either in the brainstem autonomic centers or in the periphery are responsible (Ernsberger et al., 1999).

In contrast to the "I1R hypothesis", it has been proposed that all of the therapeutic actions of moxonidine and other imidazolines can be entirely accounted for by their activity at α2AR (Zhu et al., 1999; Szabo et al., 2002). This conclusion is based almost entirely on studies of centrally mediated cardiovascular responses. Because both I1R and α2AR can mediate comparable sympathoinhibitory responses, separation of their actions is difficult, even using mutant mouse models (Zhu et al., 1999; Tolentino-Silva et al., 2000). In the present study, we examined metabolic responses to I1R and α2AR agonists, given the possibility that responses to these two classes of receptor may not be identical in all respects.

The present study was thus designed as an indirect test of four competing hypotheses regarding the metabolic effects of antihypertensives. Each hypothesis makes specific predictions regarding the effectiveness of the three comparison agents. According to the hemodynamic hypothesis, all three agents should improve glucose and lipid metabolism by decreasing peripheral vascular resistance. The sympathoadrenal hypothesis predicts beneficial effects of the sympathoinhibitory agents moxonidine and α-methyldopa, whereas hydralazine, by eliciting reflex sympathoexcitation, should impair metabolic homeostasis. The I1R hypothesis predicts unique beneficial effects of the I1R agonist moxonidine. The α2AR hypothesis predicts identical responses to treatment with the two α2AR agonists moxonidine and α-methyldopa, especially because the active metabolite of α-methyldopa, α-methylnorepinephrine, has nearly identical affinity for α2AR as moxonidine (Ernsberger et al., 1993). Thus, we compared the effects of moxonidine, α-methyldopa, and hydralazine on blood pressure and circulating glucose, insulin, insulin C-peptide, glucagon, triglycerides, cholesterol, and free fatty acids in the fasted state and after a glucose load.

Materials and Methods

Materials. Moxonidine was provided by Solvay Pharmaceuticals (Hannover, Germany). α-Methyldopa and hydralazine were obtained from Sigma-Aldrich (St. Louis, MO).

Animal Procedures. Adult male and female SHROB were used in these studies. Animals were housed individually and were provided food (Teklad 8664; Teklad, Madison, WI) and water ad libitum before drug treatment. Animals were on a 12:12-h light/dark cycle (lights on from 7:00 AM to 7:00 PM) and were maintained at a constant temperature of 21°C. These procedures were carried out with the approval of the Case Western Reserve University Animal Care and Use Committee.

Chronic Drug Treatment. All drugs were dissolved in 0.1% citric acid. SHROB were administrated drugs orally for 15 days by admixture with powdered rat chow (identical formulation to standard chow) before pelleting. Food intake was allowed ad libitum and monitored continuously so that adjustments could be made to drug concentrations as necessary. Moxonidine, a mixed I1R/α2AR agonist, was administrated at 4 mg/kg/day. Preliminary studies indicated that this dose was as effective as the previously used 8 mg/kg/day (Ernsberger et al., 1996). α-Methyldopa, an α2AR agonist, was given at a dose of 200 mg/kg/day shown to be effective in SHR (Tabei et al., 1970). Hydralazine, a direct vasodilator, was given at a dose of 10 mg/kg/day, which had previously been shown to control hypertension in the SHR model (Sabatin et al., 2001). Control untreated SHROB were fed normal chow. Body weight and food intake were determined every other day. After 12 days of treatment, tail cuff blood pressure was determined between 12:00 PM and 4:00 PM after habituation of the animals to the testing procedure. Systolic blood pressures were averaged between three and five measurements in a single session separate from the habituation sessions. After 15 days of treatment, an oral glucose tolerance test (OGTT) (6 g/kg glucose by oral gavage) was conducted after an 18-h fast.

OGTT. As described previously (Ernsberger et al., 1999), rats were given by oral gavage a 50% glucose solution at a dose of 6 g/kg body weight. Blood (0.2 ml) was obtained from the tail of conscious animals at baseline and 30, 60, 120, 240, and 360 min after the glucose load. Plasma glucose, insulin, and C-peptide were determined at each time point. Plasma glucagon and FFA were determined at 0, 30, and 60 min only. Area under the curve (AUC) was determined for each parameter for quantification of the OGTT.

Plasma Biochemical Measurements. Blood samples were chilled on ice, centrifuged for 20 min at 5000g at 4°C, and the plasma frozen at −70°C until assayed for plasma glucose, insulin, C-peptide, glucagon, triglyceride, total cholesterol, and FFA. Plasma glucose was determined by colorimetric glucose oxidase assay (Sigma-Al drich). Plasma insulin, C-peptide, and glucagon radioimmunoassay kits were used with rat insulin, C-peptide, and glucagon standards and antibodies directed against rat insulin, C-peptide, and glucagon,
Results

Food Intake, Body Weight, and Blood Pressure. Average food intake did not differ between groups (Table 1). Change in body weight was identical between moxidine, hydralazine, and untreated control groups, but the α-methyldopa group lost a slight amount of weight despite the lack of change in food intake.

Figure 1 shows systolic blood pressure as determined by tail cuff in treated and untreated control SHROB. Each of the agents reduced blood pressure nearly to normotensive levels, defined as systolic pressure <150 mm Hg. Note that all three antihypertensive agents were equally effective at reduction of blood pressure in the SHROB model (p < 0.001, one-way ANOVA).

Metabolic Parameters in the Fasted State. Table 1 presents the metabolic characteristics in treated SHROB and untreated SHROB. The SHROB exhibits the plasma markers of metabolic syndrome X, including hyperinsulinemia, hyperglucagonemia, and type IV hyperlipidemia. Treatment with 4.0 mg/kg/day moxidine significantly reduced fasting plasma insulin, glucagon, triglycerides, total cholesterol, and FFA compared with untreated SHROB. α-Methyldopa tended to reduce fasting insulin, but this effect was not significant (p > 0.05). α-Methyldopa did not reproduce any of the metabolic effects seen after moxidine treatment. Hydralazine significantly reduced fasting plasma glucagon and FFA compared with untreated SHROB, but had no other effect on fasting metabolic parameters.

Response to a Glucose Load. Fig. 2A shows plasma glucose in treated and untreated SHROB at various times after the glucose load. Consistent with previous results, SHROB showed glucose intolerance with fasting normoglycemia. Moxonidine significantly reduced the glucose response compared with untreated SHROB. However, α-methyldopa and hydralazine further impaired the glucose response compared with untreated SHROB (p < 0.05). Figure 2B shows the glucose AUC in treated and untreated SHROB. Moxonidine significantly reduced the glucose AUC compared with untreated SHROB (13.6 ± 2.4 versus 42.5 ± 9.9 g · min/dl). α-Methyldopa and hydralazine increased the glucose AUC compared with untreated SHROB (68 ± 26 and 110 ± 21 g · min/dl, respectively; p < 0.001, one-way ANOVA).

Figure 3A illustrates the plasma insulin response in treated and untreated SHROB during an OGTT. The observed levels of insulin are 20-fold elevated compared with lean SHR controls (Velliquette et al., 2002). Moxonidine significantly facilitated the initial glucose induced insulin response at the first two time points compared with untreated SHROB (p < 0.001). In contrast, α-methyldopa and hydralazine significantly blunted the insulin response during the first 120 min. Interestingly, the group treated with hydralazine showed a significant fall in plasma insulin during the first 240 min after a glucose load (p < 0.001). Figure 3B represents the plasma insulin AUC in treated and untreated SHROB. Moxonidine significantly increased the insulin AUC.

TABLE 1
Fasting plasma markers for metabolic syndrome X in treated and untreated SHROB

<table>
<thead>
<tr>
<th></th>
<th>Untreated SHROB (N = 14)</th>
<th>Moxonidine-Treated SHROB (N = 15)</th>
<th>α-Methyldopa-Treated SHROB (N = 12)</th>
<th>Hydralazine-Treated SHROB (N = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight change (g)</td>
<td>12.3 ± 4.1</td>
<td>9.4 ± 4.5</td>
<td>−15.0 ± 5.3*</td>
<td>11.2 ± 3.5</td>
</tr>
<tr>
<td>Average food intake (g/day)</td>
<td>27.1 ± 0.5</td>
<td>29.8 ± 0.8</td>
<td>26.8 ± 1.6</td>
<td>30.5 ± 1.1</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>112 ± 5.4</td>
<td>105 ± 4.1</td>
<td>111 ± 6.7</td>
<td>107 ± 3.4</td>
</tr>
<tr>
<td>Insulin (ng/ml)</td>
<td>20 ± 3.7</td>
<td>11 ± 1.7*</td>
<td>11 ± 2.6</td>
<td>17 ± 3.2</td>
</tr>
<tr>
<td>C-peptide (nM)</td>
<td>3.1 ± 0.07</td>
<td>3.1 ± 0.47</td>
<td>3.8 ± 0.37*</td>
<td>3.3 ± 0.73</td>
</tr>
<tr>
<td>Glucagon (pg/ml)</td>
<td>114 ± 6.8</td>
<td>87 ± 11*</td>
<td>114 ± 8.4</td>
<td>86 ± 4.7*</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>466 ± 42</td>
<td>217 ± 17*</td>
<td>368 ± 36</td>
<td>451 ± 93</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>379 ± 31</td>
<td>273 ± 39*</td>
<td>354 ± 57</td>
<td>436 ± 67</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>1.58 ± 0.11</td>
<td>1.29 ± 0.07</td>
<td>1.42 ± 0.11</td>
<td>1.16 ± 0.02*</td>
</tr>
</tbody>
</table>

* Significantly different from untreated SHROB, p < 0.05.
compared with untreated SHROB ($p < 0.01$). This was primarily an effect on the early insulin response during the first 60 min ($700 \pm 160$ versus $13 \pm 128$ ng·min/ml, $p < 0.001$).

In contrast, chronic hydralazine significantly reduced the total plasma insulin AUC compared with untreated SHROB ($-2.3 \pm 1.0$ versus $3.9 \pm 1.8$ μg·min/ml) ($p < 0.001$).

Plasma C-peptide was measured as an index of insulin secretion, because plasma insulin reflects both the secretion and turnover of insulin. Figure 4A shows the plasma C-peptide response to the glucose load. Plasma C-peptide generally mirrored the insulin response in all groups. However, moxonidine lowered the fasting plasma insulin to C-peptide ratio, suggesting that insulin turnover was greater after moxonidine treatment compared with untreated controls (Table 1). Figure 4B illustrates the plasma C-peptide AUC for all groups. Chronic moxonidine treatment significantly increased the C-peptide AUC compared with untreated controls ($1.25 \pm 0.29$ versus $0.43 \pm 0.14$ μM·min) ($p < 0.01$). As seen with insulin, hydralazine treatment inverted the normal response of C-peptide to a glucose load, resulting in a significant negative C-peptide AUC ($-0.57 \pm 0.23$ μM·min) ($p < 0.001$). These results suggest, in part, that moxonidine improves glucose tolerance by increasing the glucose induced insulin secretion, whereas α-methyldopa and hydralazine...
worsen glucose tolerance by inhibiting the insulin secretory response to a glucose challenge.

Figure 5A shows the plasma glucagon response to the glucose load in treated and untreated SHROB. As previously reported, glucagon shows a paradoxical rise in response to a glucose load in SHROB (Velliquette et al., 2002). Moxonidine significantly reduced this abnormal plasma glucagon response at all time points postload compared with untreated SHROB ($p < 0.001$). In marked contrast, chronic treatment with $\alpha$-methyldopa potentiated the glucagon response to a glucose load by 2-fold at 30 min and nearly 3.5-fold at 60 min postload ($p < 0.001$). Hydralazine also increased the glucagon response but only at 60 min compared with untreated controls ($p < 0.05$). Plasma glucagon AUC is presented in Fig. 5B. Moxonidine significantly reduced the glucagon AUC compared with untreated SHROB ($5.0 \pm 0.6$ versus $10 \pm 1.3 \text{ ng} \cdot \text{min}/\text{ml}; p < 0.05$; one-way ANOVA). $\alpha$-Methyldopa and hydralazine significantly elevated the glucagon AUC compared with untreated SHROB ($*, p < 0.05; ***, p < 0.001$, one-way ANOVA).

Figure 4A shows the plasma C-peptide response to a 6 g/kg oral glucose load in 18-h fasted treated and untreated SHROB. Plasma C-peptide generally paralleled the insulin response. Animals treated with moxonidine showed robust glucose-induced insulin secretion compared with untreated controls. In contrast, the $\alpha$-methyldopa-treated group showed a blunted insulin response, and hydralazine treatment inverted the insulin secretory response to glucose resulting in a fall in C-peptide after the load. $*, p < 0.05; **, p < 0.01$, two-way ANOVA. C-peptide AUC in treated and untreated SHROB in response to a glucose load Moxonidine significantly increased the C-peptide AUC compared with untreated SHROB. Chronic hydralazine treatment resulted in a significantly negative C-peptide AUC. $**, p < 0.01; ***, p < 0.001$, one-way ANOVA.

Figure 5A shows the plasma glucagon response to a 6 g/kg oral glucose load in 18-h fasted in treated and untreated SHROB. Untreated SHROB show a paradoxical increase in glucagon in response to a glucose load. Moxonidine significantly reduced the glucagon response at all time points compared with untreated SHROB. Treatment with $\alpha$-methyldopa potentiated the glucagon response to a glucose load by 2-fold at 30 min and nearly 3.5-fold at 60 min postload. Hydralazine treatment elevated the glucagon response at 60 min only. $*, p < 0.05; ***, p < 0.001$, two-way ANOVA. B, glucagon AUC in treated and untreated SHROB after a glucose load. Moxonidine significantly reduced the glucagon AUC compared with untreated SHROB. $\alpha$-Methyldopa and hydralazine significantly elevated the glucagon AUC compared with untreated SHROB. $*, p < 0.05; ***, p < 0.001$, one-way ANOVA.

Figure 6 shows the percentage of change in FFA levels after an oral glucose load in treated and untreated SHROB. As previously observed, control SHROB fail to show the expected suppression of FFA after a glucose load (Velliquette et
Fig. 6. Percentage of change in plasma FFA levels after a 6 g/kg oral glucose load in 18-h fasted treated and untreated SHROB. Moxonidine significantly reduced plasma FFA levels at all time points. The α-methyl-
dopa and hydralazine treated groups did not differ from untreated SHROB. *** p < 0.001, two-way ANOVA.

Discussion

We showed that three antihypertensive agents in clinical use had contrasting effects on glucose tolerance in an insulin-
resistant rodent model, despite equal effectiveness in lowering
blood pressure. The mixed I1R/α2AR agonist moxonidine
improved plasma markers of metabolic syndrome X and improved all measured parameters of glucose tolerance. The α2AR agonist α-methyl-
dopa did not significantly improve plasma markers of metabolic syndrome X and actually worsened glucose tolerance compared with untreated controls. Hydralazine, a direct vasodilator, significantly reduced FFA, possibly reflecting improved adipose tissue insulin sensitivity. These data reinforce the hypothesis that stimulation of I1R and not sympathoinhibition increases in blood flow or activation of α2AR mediate changes in lipid metabolism after antihypertensive treatment with imidazo-
line agents such as moxonidine.

mediated effects associated with individual agents. Thus, the
improved glucose tolerance induced by moxonidine may be
mediated by specific receptors.

Based on current theories regarding the metabolic actions
of antihypertensive agents, we identified four possible hy-
potheses, each predicting a different outcome in our tests of
three different agents. The hemodynamic hypothesis pre-
dicted that each of the three agents would improve glucose
disposal equally by lowering peripheral resistance and im-
proving glucose and insulin delivery to active tissues. Be-
cause only one of the agents was effective and the other two
actually worsened glucose tolerance, the hemodynamic hy-
pothesis cannot account for the results. The sympathoadre-
nal hypothesis, which postulates a primary role for the sym-
pathoadrenal system in the control of glucose metabolism,
predicts that the two sympathoinhibitory agents moxonidine
and α-methyl-
dopa would improve glucose tolerance. Only
moxonidine was effective, whereas α-methyl-
dopa actually
worsened glucose tolerance, which is not consistent with an
overriding importance of sympathoadrenal inhibition. None-
theless, the sympathoadrenal hypothesis correctly predicts
the deleterious effect of hydralazine on glucose disposal, be-
cause this agent is known to evoke reflex sympathoadrenal
activation. Thus, the present data are consistent with con-
tributory role of sympathoadrenal activity in the effects of
some antihypertensive agents on glucose tolerance. The
mechanisms for improvements in glucose metabolism after
inhibition of sympathetic tone have been recently reviewed.
Reduced elevated sympathetic outflow might improve glu-
cose tolerance by diminishing the actions of catecholamine
to decrease insulin and increase glucagon secretion, stimulate
glycogenolysis and gluconeogenesis, elevate FFA, and de-
ncrease skeletal muscle blood flow (Ernsberger et al., 1998).

The hypothesis most consistent with the data is the imi-
dazoline receptor hypothesis, which proposes that direct ac-
tivation of imidazoline receptors by moxonidine mediates the
actions of this agent on glucose metabolism. A limitation of
the present study is that we did not demonstrate blockade of
moxonidine’s effects with antagonists. Preliminary studies
using osmotic minipumps to deliver drugs continuously indi-
cated that doses of efaxoroxan high enough to antagonize the
actions of moxonidine had effects when given alone (data not
shown), consistent with previous reports of improved glucose
metabolism and insulin secretion after efaxoroxan treatment
(Berridge et al., 1992). Several mechanisms for a direct in-
fluence of I1R on glucose metabolism are possible. Radioi-
gand binding studies have identified I1R in the endocrine
pancreas and liver (Ernsberger et al., 1995), and liver local-
ization has been confirmed by Northern blot analysis of the
I1R candidate gene (Piletz et al., 1999). An action of I1R in the
endocrine pancreas is the most likely explanation for the
restoration of the early phase of insulin secretion induced by
moxonidine treatment. Chronic treatment with moxonidine
also increases expression and tyrosine phosphorylation of
insulin receptor and insulin receptor substrate-1 in the liver,
possibly through direct action (Ernsberger et al., 1999). We
have recent found that an acute improvement in glucose
tolerance can be induced by moxonidine if α1-ARs are blocked
(Velliquette et al., 2003). This improvement in glucose toler-
ance induced by a single dose of moxonidine could be blocked
by efaxoroxan, implicating an I1R mechanism. Similarly, the
acute lipid-lowering actions of moxonidine are blocked by
Effects and are not affected by α2-AR blockade (Velliquette et al., 2003). Thus, based on prior results, the improvements in glucose and lipid metabolism observed in the present study after chronic treatment are mostly likely mediated by I1R.

Some studies have proposed that all of the therapeutic actions of moxonidine and other imidazolines can be entirely accounted for by their activity at α2-ARs (Zhu et al., 1999; Szabo et al., 2002). This α2-AR hypothesis is based on studies of centrally mediated cardiovascular responses, which can be mediated by either I1R or α2-AR acting in synergy. In the present study, the α2-AR hypothesis predicts that moxonidine should have effects identical to the specific α2-AR agonist α-methyladrenalinphtol. Notably, moxonidine and the active metabolite of α-methyldopa, α-methylnorepinephrine, have similar Ki values for the α2-ARs (Ernsberger, 2000). Although α-methyldopa reduced blood pressure similarly to moxonidine, the metabolic outcomes were in marked contrast, with no decreases in circulating lipids and a worsening of glucose tolerance. Deterioration of glucose tolerance seemed to be the result of an attenuated early phase of insulin release in response to a glucose load and a large increase in glucagon secretion. The most likely mechanism is activation of α2-AR on pancreatic islets, which are known to inhibit insulin and promote glucagon secretion (Hirose et al., 1992). Chronic treatment with α-methyldopa has been reported to have negative affects on glucose homeostasis in humans (Benfield and Hunter, 1982) but has not been previously tested in rats. Moxonidine, even though it is a full agonist at α2-AR, did not elicit these responses and in fact elicited opposite effects. The simplest explanation for the contrasting effects of α-methyldopa and moxonidine is that a second nonadrenergic receptor is involved in the actions of moxonidine. Thus, the α2-AR hypothesis must be rejected as an explanation for the metabolic actions of imidazolines.

Consistent with previous studies from our laboratory using higher doses of moxonidine given for a longer period of time (8 mg/kg/day for 90 days) (Ernsberger et al., 1996, 1999), we noted improvements in multiple markers of metabolic syndrome X, including improved glucose tolerance, reduced fasting levels of insulin, triglycerides, cholesterol, and FFA. However, our prior studies also found a decrease in food intake and decreased rate of weight gain. These effects on feeding and weight did not occur at the dose of 4 mg/kg/day in the present study. A limitation of our past studies was a possible independent effect of reduced food intake and body weight gain on metabolic parameters. We now show that the metabolic effects observed at the higher dose can be reproduced at 4 mg/kg/day independent of any alteration in caloric intake or body weight. The other two agents had no major impact on food intake or body weight. The small decrease in body weight induced by α-methyladrenalinphtol failed to translate into significant improvements in glucose or lipid metabolism.

Possible etiological factors underlying metabolic syndrome X have recently been identified. Cytokines such as tumor necrosis factor-α and interleukin-6 can be produced by adipocytes and may contribute to insulin resistance and metabolic syndrome X, at least in part through generation of reactive oxygen species (Fernandez-Real and Ricart, 2003). The relationship between I1R and cytokines is unknown. However, its gene candidate shares several motifs with cytokine receptors (Piletz et al., 2000) and its signaling pathway overlaps those of cytokine receptors (Ernsberger, 2000). It is not known whether I1R mediate pro- or anti-inflammatory actions. FFAs mediate inflammatory actions on the vascular endothelium that may contribute to insulin resistance (Toborek et al., 2002). By chronically lowering FFA, moxonidine may make a small contribution to reducing inflammation through this pathway. It might be argued that because the SHROB rat model is a natural occurring leptin receptor knockout, the absence of leptin signaling might have blunted or augmented the actions of imidazolines. However, similar effects of moxonidine are seen in the fructose fed and lean SHR models (Ernsberger et al., 1996; Rosen et al., 1997), which have intact leptin receptors.

A recent study found improved insulin sensitivity during acute hydralazine infusion in anesthetized SHR rats, which was correlated with increased skeletal muscle blood flow (Pitre et al., 1999). The results were interpreted as supporting the hemodynamic hypothesis, which attributes the insulin resistance associated with hypertension to impaired delivery of blood to metabolically active tissues. In the present study, we found that chronic hydralazine treatment of obese animals form the same SHR genetic background not only failed to increase insulin sensitivity but also actually decreased it. The apparently contrasting effects of acute and chronic hydralazine may reflect the contribution of reflex sympathetic activation, which might be blunted in acute surgical preparations. Reflex stimulation of catecholamine release has been reported after hydralazine treatment in rats (Sanbar and de Romero, 1969). In humans, reflex sympathetic activation acutely induces insulin resistance (Jamerston et al., 1993).

In agreement with many clinical studies, blood pressure-lowering agents do not necessarily affect comorbidities associated with hypertension such as glucose intolerance and hyperlipidemia. In fact, therapy for hypertension with a direct vasodilator actually worsens glucose metabolism in the SHROB model of metabolic syndrome X. Furthermore, reduced sympathetic outflow does not seem necessary for the therapeutic outcomes because α-methyladrenalinphtol did not reproduce any of the benefits observed after moxonidine treatment. The results of this study suggest but do not establish that the I1R component of moxonidine is responsible for the therapeutic benefits after chronic moxonidine treatment. The ability of moxonidine to activate α2-AR may limit its effectiveness in the treatment of glucose intolerance and insulin resistance. Development of more specific agonists at I1R may lead to improved therapeutic agents for the treatment of metabolic syndrome X.

Acknowledgments

We thank Robin Mooney for her dedicated technical assistance.

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