Specific inhibition of Hormone-Sensitive Lipase Improves Lipid Profile while Reducing Plasma Glucose

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ABSTRACT

Elevation of plasma free fatty acids has been linked with insulin resistance and diabetes. Inhibition of lipolysis may provide a mechanism to decrease plasma fatty acids, thereby improving insulin sensitivity. Hormone-sensitive lipase (HSL) is a critical enzyme involved in the hormonally regulated release of fatty acids and glycerol from adipocyte lipid stores, and its inhibition may thus improve insulin sensitivity and blood glucose handling in type 2 diabetes. In rat adipocytes, forskolin-activated lipolysis was blocked by in vitro addition of a potent and selective HSL inhibitor or by prior treatment of the animals themselves. Antilipolytic effects also were demonstrated in overnight-fasted mice, rats, and dogs with species-dependent effects on plasma free fatty acid levels but with similar reductions in plasma glycerol being observed in all species. Inhibition of HSL also reduced hyperglycemia in streptozotocin-induced diabetic rats. The data support a connection between adipose tissue lipolysis and plasma glucose levels.

Elevated plasma levels of free fatty acids (FFAs) are thought to play a major role in the pathogenesis of insulin resistance and type 2 diabetes (Unger, 1995; Boden, 1997; Frayn, 2002) by inhibiting glucose uptake and utilization by muscle (Kim et al., 2001) and causing increased glucose output by the liver (Large and Arner, 1998). These events combine to produce the hyperglycemia that is characteristic of type 2 diabetes (Groop et al., 1991). The increases in plasma FFA levels are the result of increased mobilization from adipose tissue. Hormone-sensitive lipase (HSL), thought to be the rate-limiting enzyme in adipose tissue lipolysis, hydrolyzes the stored triglycerides into monoglycerides and FFA (Large and Arner, 1998).

The activity of HSL is acutely activated via cAMP and protein kinase A-mediated phosphorylation (Anthonsen et al., 1998) in response to epinephrine/norepinephrine. Insulin stimulation of adipocytes prevents HSL activation, leading to a decrease in the release of FFA and glycerol (Langin et al., 1998). The role of elevated plasma FFA in type 2 diabetes has led to the proposal that HSL may be a potential therapeutic target for this disease, lowering plasma FFA levels and thereby reducing insulin resistance. Results with HSL knockout mice, however, have raised the question of whether it is a suitable target. Conflicting effects on both insulin sensitivity (Osuga et al., 2000; Saltiel, 2000; Wang et al., 2001; Haemmerle et al., 2002; Okazaki et al., 2002; Voshol et al., 2003) and insulin secretion (Roduit et al., 2001; Mulder et al., 2003) have been reported.

Small-molecule HSL inhibitors have recently been identified (Vertesy et al., 2002; Slee et al., 2003; De Jong et al., 2004; Ebdrup et al., 2004; Lowe et al., 2004). Here, we describe a highly potent, reversible, and selective inhibitor and use it to clarify the effects of HSL inhibition in isolated adipocytes and on the levels of glycerol, FFA, and glucose in mouse, rat, and dog.

ABBREVIATIONS: FFA, free fatty acid; HSL, hormone-sensitive lipase; NEFA, nonesterified fatty acid; BAY, 4-isopropyl-3-methyl-2-[(3S)-3-methylpiperidin-1-yl] carbonyl]isoxazol-5(2H)-one; MOPS, 4-morpholinepropanesulfonic acid; BAY G, 4-bromo-N,3-dimethyl-5-oxo-N-phenylisoxazol-2(SH)carboxamide; BAY P, N,N,3-trimethyl-5-oxo-4-(phenylthio)isoxazol-2(SH)carboxamide; GLP, glucagon-like peptide.
Materials and Methods

Materials. Pancreatic lipase, lipoprotein lipase, acetylcholinesterase, bovine serum albumin, lipase substrate, and Ficoll were obtained from Sigma-Aldrich (St. Louis, MO). Collagenase was obtained from Serva (Heidelberg, Germany). N-Acyl-peptide hydrolyase was obtained from Roche Diagnostics (Indianapolis, IN). N-Acetyl-methionine-aminomethylcoumarine was obtained from Bachem California (Torrance, CA). [3H]Triolein was obtained from GE Healthcare (Little Chalfont, Buckinghamshire, UK). NEFA test kit was obtained from Wako Bioproducts (Richmond, VA). The triglyceride test kit (TPO-Grinder) was obtained from Sigma-Aldrich. The rat insulin enzyme immunoassay kit was obtained from ALPCO Diagnostics (Windham, NH). Human adipocyes were obtained from Zebio, Inc. (Research Triangle Park, NC).

Synthesis of HSL Inhibitor. Chemically, BAY (Fig. 1) is a 5-(2H)-isoxazolonyl urea, produced as a low-melting crystalline solid. The parent heterocycle is synthesized from the substituted acetocetate ester, followed by acylation and urea formation. Details of the synthesis and the structure-activity relationships of a variety of similar isoxazolone HSL inhibitors are detailed in a separate publication (Lowe et al., 2004).

In Vitro Enzyme Assays. Compound inhibition of enzyme activity was measured for the following: hormone-sensitive lipase (human recombinant), hepatic lipase (rat purified; Kresse et al., 1991), pancreatic lipase, lipoprotein lipase, acetylcholinesterase, and N-acetyl-peptide hydrolyase.

Inhibition of HSL is determined by incubating for 1 h at room temperature in buffer containing 27.5 mM MOPS, pH 7.2, 55 mM KCl, 0.1% bovine serum albumin, 5% ethanol, 0.25% dimethylsulfoxide, 150 μM tricaprin, and 12 nM enzyme. The reaction is then quenched by the addition of 0.2 volumes of 5 mM butyrobromide, and the concentration of nonesterified fatty acid determined colorimetrically using a NEFA kit according to manufacturer’s instructions. The data were analyzed with Prism 3.0.3 (GraphPad Software Inc., San Diego, CA) to determine the IC50.

Inhibition of human pancreatic lipase is determined by incubating for 40 min at room temperature in buffer containing 25 mM Tris-HCl, pH 8.0, 0.25% dimethylsulfoxide, 0.17 U of enzyme, and lipase substrate diluted 1:300. The reaction is then quenched by the addition of 0.2 volumes of 5 mM butyrobromide, and the concentration of nonesterified fatty acid determined colorimetrically using a NEFA kit according to manufacturer’s instructions. The data were analyzed with Prism 3.0.3 (GraphPad Software Inc.) to determine the IC50 with BAY demonstrating an IC50 > 10 μM.

Inhibition of hepatic lipase is determined by incubating 15 μM enzyme with substrate (25 μM [3H]tri olein at 285 μCi/mmol) at 37°C for 30 to 60 min in buffer containing 0.17 M Tris HCl, pH 8.5, 1 M NaCl, and 0.17% bovine serum albumin. The reaction (0.2 ml) is stopped with a mixture (3.3 ml) of methanol:chloroform:heptane (1:41:1.25:1). Oleic acid is extracted at pH 10.5 as described (Briquet-Laugier et al., 1999) and quantitated by liquid scintillation counting.

Reversibility of HSL Inhibition. For reversibility studies, two compounds closely related to BAY were used: BAY G with an IC50 of 6 nM and BAY P with an IC50 of 200 nM. Hormone-sensitive lipase at 1.1 μM was incubated at room temperature for 30 min with 10 μM BAY G, 10 μM BAY P, or with vehicle alone (0.1% dimethyl sulfoxide). Aliquots of the incubation were denoted as no column, postcolumn + compound. The no column samples were kept on ice. The postcolumn samples were applied to a G25 spin column to remove compound. The column + compound were applied to a spin column to remove compound, then compound was added back to a final concentration of 10 μM. All samples were then assayed for hormone-sensitive lipase activity as described above. Results are expressed as average OD560± S.D.

Adipocyte Lipolysis. Lipolysis was measured in 3T3-L1 cells differentiated for 8 days (Fong, 1990), in freshly isolated rat adipocytes prepared as described (Rodbell, 1964), or in human adipocytes. The adipocytes were washed in buffer containing 3% bovine serum albumin and then preincubated in compound for 30 min at 37°C. Forskolin was then added to 5 to 50 μM for 1h at 37°C. At the end of this incubation, the lipolysis rate was determined by measuring the amount of fatty acids (3T3-L1 cells) or glycerol (adipocytes) released into the medium using a NEFA kit or the triglyceride (TPO-Grinder) kit, respectively. The data were analyzed with Prism 3.0.3 to determine the IC50.

Animals. Male Balb/C mice were obtained from Charles River Laboratories, Inc. (Wilmington, MA), whereas male Wistar rats were obtained from Harlan (Indianapolis, IN). The animals were fed ad libitum until the day before the experiment when the food was removed overnight (16–18-h fast). Male beagle dogs from the in-house colony were also fasted overnight. Male Sprague-Dawley rats (Charles River Laboratories, Inc.) were made diabetic by tail vein injection of 45 mg/kg streptozotocin in citrate buffer, pH 4.0. One week later, they were fasted for 5 h prior to the experiment.

In Vivo Testing. Overnight-fasted Balb/C mice and Wistar rats were given an oral dose of 30 mg/kg BAY or 0.5% methylcellulose. Blood was collected by retroorbital puncture just prior to dosing and again at 0.5, 1, 1.5, and 2 h after dosing the mice. Blood was collected from rats by tail vein clip prior to dosing and at 1, 2, and 3 h after dosing. The streptozotocin diabetic rats were tail-blinded after 3 h. The dogs were given BAY or polyethylene glycol 400/propanol glycol (80:20) via oral gavage. A blood sample was withdrawn from a cephalic vein just prior to dosing and after 1, 2, 4, and 6 h. The dogs were dosed for both the vehicle and BAY treatments, which were done 1 week apart from each other. Plasma was analyzed for free fatty acid and glycerol concentration using a NEFA kit and the triglyceride (TPO-Grinder) kit, respectively.
Statistics. The results are expressed as mean ± S.E.M. Intergroup comparisons were performed by unpaired Student’s t test or by analysis of variance with Dunnett’s t test for multiple comparisons, where appropriate. P < 0.05 was considered significant.

Insulin Secretion from Rat Islets. Rat pancreatic islets were isolated as follows. Briefly, the rat was anesthetized with 0.2 ml Nembutal/100 g body weight. The pancreas was inflated with 15 to 20 ml of Hanks’ solution (127 mM NaCl, 5.4 mM KCl, 0.34 mM Na2HPO4, 4.4 mM KH2PO4, 20 mM HEPES, 1.2 mM CaCl2, and 5 mM glucose, pH 7.4). The pancreas was then cut into small pieces, rinsed with Hanks’ solution, and digested for 10 min with 2.3 mg/ml collagenase in Hanks’ solution at 37°C. The tissue was then rinsed with Hanks’ solution to remove the collagenase, and the tissue was put into a Ficoll gradient consisting of layers of 27, 23, 20.5, and 11% Ficoll. Following centrifugation for 10 min at 1600 rpm at room temperature, the islets are removed from the interface of the 11 and 20.5% Ficoll layers, rinsed in Hanks’ solution, and hand-picked using a Pasteur pipette under a microscope. For the measurement of insulin secretion, five islets were loaded per tube in 2 ml of incubation buffer (115 mM NaCl, 5 mM KCl, 24 mM NaHCO3, 3 mM glucose, 0.22 mM CaCl2, 0.1 mM MgCl2, 0.025% bovine serum albumin, and 2 mM HEPES, pH 7.4). After 30-min preincubation, islets were then transferred into incubation buffer containing different secretagogues and 1 μM compound, as listed under Results, for 60 min. The media were removed for the determination of insulin using the 1-2-3 Rat Insulin enzyme immunoassay kit.

Results

In Vitro Characterization of the HSL Inhibitor. BAY inhibited human hormone-sensitive lipase with an IC50 of 5 nM (Fig. 2). The compound showed no significant activity at concentrations up to 10 μM against the mechanistically related enzymes hepatic lipase, pancreatic lipase, lipoprotein lipase, acetylcholinesterase, and N-acyl-peptide hydrolase (data not shown). Although the IC50 is time-dependent, reversibility was demonstrated by the removal of compound either by dialysis or, more rapidly, through the use of a spin column (Fig. 3). This kinetic behavior was confirmed in a comparison of the compound with a structurally distinct series of inhibitors (Ebdrup et al., 2004). The mechanism of inhibition was shown to be uncompetitive.

Inhibition of Adipocyte Lipolysis. In 3T3-L1 adipocytes, BAY inhibited forskolin-stimulated lipolysis 90% with an IC50 of 40 nM (Fig. 4A), whereas it inhibited basal lipolysis only 30% with an IC50 of 60 nM (Fig. 4B). BAY was equally effective in inhibiting forskolin-stimulated lipolysis in rat (Fig. 4C) and human (Fig. 4D) adipocytes with EC50 values of 30 and 40 nM, respectively. Because the compound was effective in inhibiting lipolysis in adipocytes, we next determined the effect of compound on lipolysis in vivo through measurement of plasma FFA and glycerol.

In Vivo Inhibition of Lipolysis. The effect of BAY on plasma free fatty acid and glycerol levels were determined in mice, rats, and dogs following an overnight fast to stimulate lipolysis (Fig. 5). BAY reduced plasma glycerol levels by 50% in all three species. However, BAY caused a much greater reduction in plasma free fatty acid levels in dogs (70%) than in mice (25%) and had no significant effect in rats. An equivalent dose of acipimox, a niacin derivative used to reduce plasma lipids through inhibition of lipolysis, reduced plasma glycerol levels in rats to the same extent as BAY, but it was less effective than BAY in mice (25 versus 50%). In contrast to BAY, acipimox inhibited free fatty acid levels by 90% in rats, whereas in mice it was less effective than was BAY.

Ex Vivo Inhibition of Lipolysis. Following treatment of normal rats with BAY, plasma glycerol (Fig. 6A) remained suppressed after 8 h despite the absence of measurable concentrations of BAY in plasma (Fig. 6B). To determine whether the sustained suppression of glycerol levels could be attributed to inhibition of adipocyte lipolysis or was due to off target effects of the compound, animals were treated and the rate of lipolysis determined at several time points. The inhibitory effect of BAY was still observed as a blunted forskolin response in omental fat removed at 4 and 8 h (Fig. 6C). Thus, the in vitro BAY-induced suppression of lipolysis in the presence of forskolin was preserved in omental fat from BAY-treated rats. These data may explain the sustained decrease in plasma glycerol against the progressive loss of circulating BAY. In other studies, no evidence was found for the generation of active metabolites of BAY in the plasma (data not shown). Taken together, these results suggest that BAY is
sequestered in fat depots following administration and remains efficacious in inhibiting adipocyte lipolysis.

**Effect of Acipimox and BAY in Streptozotocin Diabetic Rats.** It has been reported previously that acipimox lowers plasma glycerol and FFA in STZ rats, resulting in a decrease in plasma glucose. The effects of BAY and acipimox were compared in streptozotocin-induced diabetic rats 3 h after a single dose (Fig. 7). Both compounds lowered plasma glucose and glycerol levels, with acipimox showing a trend toward greater reduction than BAY. Acipimox significantly reduced free fatty acid levels, whereas the reduction of free fatty acids by BAY was not significant. Neither compound had any significant effect on weight gain.

**Effects on Insulin Secretion.** The changes observed in plasma glucose in response to BAY could be due to effects on insulin sensitivity or on insulin secretion. Changes in insulin secretion have been reported in animals lacking HSL. To determine whether treatment with BAY reproduced these effects, islets were isolated from rat pancreas and insulin secretion measured following treatment with various agents in the presence or absence of BAY.

BAY reduced forskolin-stimulated insulin secretion from isolated rat islets (Table 1). BAY also reduced the stimulation of glucose-dependent insulin secretion caused by other agents acting through the cAMP pathway, such as glucagon-like peptide-1. BAY further inhibited the enhancement of insulin secretion caused by several agents working through other signaling pathways, such as acetylcholine. However, BAY did not inhibit insulin secretion caused by protein kinase C activation by phorbol 12,13-dibutyrate, suggesting its role in inhibiting acetylcholine stimulation of insulin secretion is upstream of protein kinase C.

BAY had no effect on arginine or on agents acting directly on the potassium channel, such as glibenclamide. Taken together, the data support a role for HSL in the enhancement of glucose-dependent insulin secretion but not in actual nu-
trient sensing. Under no conditions did inhibition of HSL by BAY cause an enhancement of insulin secretion. The glucose-lowering effects of BAY must, therefore, be due to effects on insulin sensitivity rather than insulin secretion.

**Discussion**

We report the characterization of a potent, selective, and reversible inhibitor of HSL and have investigated its effects on adipocyte lipolysis in vitro and in vivo and on blood glucose levels in vivo. In adipocytes, BAY effectively blocked forskolin-stimulated lipolysis but did not completely eliminate it even at concentrations of BAY that were almost 250-fold greater than its IC50 value against the isolated enzyme. Similar effects were observed in adipocytes from HSL knockout mice upon stimulation of lipolysis by β3 adrenergic agonists (Wang et al., 2001) or isoproterenol (Okazaki et al., 2002). On the other hand, BAY was less potent (70 versus 32 nM) and less efficacious (30 versus 90%) in reducing basal lipolysis than forskolin-stimulated lipolysis. These results are consistent with basal lipolysis still being present in adipocytes from HSL knockout mice (Osuga et al., 2000; Saltiel, 2000; Okazaki et al., 2002), and with basal lipolysis being carried out by an enzyme distinct from HSL. Together, our results support the suggestion that another lipase with similar functionality is present in adipose tissue (Osuga et al., 2000; Saltiel, 2000; Okazaki et al., 2002). The accumulation of diglycerides in adipose tissue of HSL knockout mice (Haemmerle et al., 2002) suggests that both HSL and a putative lipase are able to hydrolyze triacylglycerol, but only HSL is capable of hydrolyzing diacylglycerol. The recently

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**Table 1**

Inhibition of insulin secretion from isolated rat islets by BAY

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Without BAY</th>
<th>With BAY</th>
</tr>
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<tbody>
<tr>
<td>3 mM glucose</td>
<td>1.30 ± 0.10</td>
<td>1.29 ± 0.22</td>
</tr>
<tr>
<td>8 mM glucose</td>
<td>1.72 ± 0.19</td>
<td>1.34 ± 0.06</td>
</tr>
<tr>
<td>15 mM glucose</td>
<td>4.51 ± 0.33a</td>
<td>3.75 ± 0.23a</td>
</tr>
<tr>
<td>100 nM GLP-1/8 mM glucose</td>
<td>2.38 ± 0.23b</td>
<td>1.53 ± 0.15b</td>
</tr>
<tr>
<td>1 mM forskolin/8 mM glucose</td>
<td>3.83 ± 0.35c</td>
<td>2.41 ± 0.43c</td>
</tr>
<tr>
<td>1 mM glybenclamide/8 mM glucose</td>
<td>3.16 ± 0.50b</td>
<td>3.35 ± 0.62b</td>
</tr>
<tr>
<td>15 mM arginine/8 mM glucose</td>
<td>2.64 ± 0.34b</td>
<td>2.49 ± 0.41b</td>
</tr>
<tr>
<td>0.1 mM acetylcholine/8 mM glucose</td>
<td>3.03 ± 0.40b</td>
<td>1.95 ± 0.27b</td>
</tr>
<tr>
<td>1 mM PDBu/8 mM glucose</td>
<td>2.80 ± 0.68b</td>
<td>2.51 ± 0.87b</td>
</tr>
</tbody>
</table>

PDBu, phorbol 12,13-dibutyrate.  
*P < 0.05 compared with islets incubated in 3 mM glucose.  
*P < 0.05 compared with islets incubated in 8 mM glucose only.  
*P < 0.05 compared with islets incubated with the same secretagogues but without BAY.
identified adipose triglyceride lipase has been proposed as a candidate for this non-HSL lipolytic activity, and selective HSL inhibitors may provide useful tools to further clarify its role (Zimmermann et al., 2004).

We examined the effects of BAY in vivo on plasma levels of glycerol and FFA. BAY reduced plasma levels of glycerol to the same extent in mice, rats, and dogs, but the effects on plasma FFA levels were species dependent with BAY being more effective in dogs than in mice and with no significant effect in rats. The results suggest that HSL is the major, if not the only, lipase involved in lipolysis in dogs. In rats, the inhibition of HSL-mediated lipolysis is sufficient to prevent the release of glycerol into the circulation but not to prevent the release of FFA. This is consistent with the finding in knockout mice that HSL is critical for diacylglycerol but not triacylglycerol hydrolysis (Haemmerle et al., 2002). Acipimox had a similar effect on plasma glycerol but a much greater effect on plasma FFA levels than BAY in rats. Acipimox exerts its anti-lipolytic effects through a G-protein-coupled receptor, HM74A (Tunaru et al., 2003), which suggests that activation of HM74A in rats leads to modulation of the activities of lipolytic enzymes other than HSL, thereby having a greater effect on lipolysis than can be accomplished solely through direct inhibition of HSL.

Initial studies on the effect of decreased plasma FFA levels on plasma glucose were carried out with acipimox and nico
tinic acid. Treatment of STZ diabetic rats with these agents resulted in a dramatic decrease in FFA and a significant decrease in plasma glucose (Reaven et al., 1988). In the present study, BAY significantly decreased plasma glycerol without a statistically significant change in FFA, and these changes were accompanied by a small, but significant, decrease in plasma glucose levels in the STZ rats. The fact that acipimox had greater effects on plasma FFA, glycerol, and glucose is consistent with the suggestion that this drug effects FFA and glycerol release through mechanisms in addition to HSL inhibition.

Plasma glucose lowering can be achieved either through improvements in insulin sensitivity or enhanced insulin secretion. To better understand the mechanism of glucose lowering, we studied the effects of BAY on insulin secretion. The expression of HSL in pancreatic β-cells has been confirmed in isolated islets from rodents (Mulder et al., 1999), as well as several β-cell lines at levels of mRNA, protein, or enzyme activity. It is generally agreed that HSL plays an important role in the lipid metabolism of pancreatic β-cells. However, whether HSL is essential for insulin release stimulated by nutrients and various secretagogues is still under investigation.

The role of HSL in insulin secretion has been studied mostly through the use of knockout mice. It has been reported that there was a defect in insulin secretion in HSL null mice (Roduit et al., 2001), whereas another study observed an intact insulin release of pancreatic islets isolated from HSL null mice (Mulder et al., 2003). Whether HSL participates in the mechanism of glucagon-like peptide (GLP)-1 insulino
tropic effect is also an uncertain issue. GLP-1 enhances insulin release via increasing cAMP and PKA pathway, which activate HSL. It has been reported that GLP-1 stimulates lipolysis in clonal pancreatic β-cells (HIT). The GLP-1-induced lipolysis might have a positive impact on insulin release via lipid signaling since lipase inhibition with orlistat substantially reduced the effect on GLP-1 on insulin release from islets of Sprague-Dawley rats (Cunningham et al., 2003). Orlistat also inhibited forskolin-stimulated insulin release from rat islets (Mulder et al., 2004). However, GLP-1 appears to have no effect on mouse islet lipolysis, and GLP-1-stimulated insulin release was intact in isolated islets from HSL null mice (Peyot et al., 2004), suggesting that HSL might not participate in the mechanism of GLP-1 insulino
tropic effect, at least in mouse islets. Whether these controversal observations may be explained by differences among animal species is yet unknown.

In the present study, we observed that BAY inhibited insulin release in response to secretagogues acting through the cAMP pathway, such as glucagon-like peptide-1 and forskolin. Interestingly, BAY also inhibited the protein kinase C pathway at a point prior to activation of protein kinase C because it was able to block responses to acetylcholine but not to phorbol 12,13-dibutyrate. BAY also showed no effect on stimulation of insulin secretion by glucose or arginine or in response to activation of K_{ATP} channel by sulfonylureas. Thus, BAY’s inhibitory effect on GLP-1 and acetylcarnitine warrants further investigation. Considering that there might be other lipases in pancreatic β-cells that might be involved in lipid signaling of insulin release, studies on the effect of BAY on islet lipolysis need to be conducted to confirm that the effect of BAY on insulin release is due to inhibition of HSL-mediated lipolysis. Furthermore, it has been reported that overexpression of HSL induces pancreatic β-cell lipotoxicity (Winzell et al., 2003). Exploring whether HSL activity is increased in diabetic pancreatic islets will help in evaluating the therapeutic potential of HSL inhibitors for type 2 diabetes. Overall, our insulin release data indicate that BAY has no insulino
tropic effect but rather an inhibitory effect on insulin secretion from isolated rat islets in response to certain secretagogues. These data support the hypothesis that BAY lowers plasma glucose through improving insulin sensitivity, not via insulin secretion.

In summary, our data support a connection between adipose tissue lipolysis and plasma glucose levels (Saloranta and Groop, 1996; Boden, 1999) in agreement with the hypothesis that decreased plasma FFAs lead to improved insulin sensitivity. However, because both glycerol and glucose were significantly decreased by BAY in the STZ rat, and FFAs were not, glucose may correlate more closely with plasma glycerol than FFA levels. This correlation raises the issue of which product of lipolysis plays a larger role in the elevated blood glucose observed in this diabetic animal model. Determination of the therapeutic potential of HSL inhibitors will require additional studies to determine what effects HSL inhibitors may have in addition to lowering FFA, glycerol, and glucose levels. The enzyme is found in numerous other tissues, where it can also serve as a neutral cholesteryl ester hydrolase. The accumulation of lipid droplets, likely consisting of cholesterol esters, have been observed in the adrenal cortex of HSL knockout mice resulting in a decrease in adrenocorticotropin-stimulated cortisol release (Li et al., 2002). The utility of HSL inhibition in diabetes treatment will depend on the presence or absence of adverse effects of HSL inhibition in tissues beyond the adipocyte.
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