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Anti-lipolytic Activity of a Novel Partial A_1 Adenosine Receptor Agonist Devoid of Cardiovascular Effects: Comparison with Nicotinic Acid

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List of nonstandard abbreviations:

CVT-3619: (2-{6-[((1R,2R)-2-hydroxycyclopentyl)amino]purin-9-yl}(4S,5S,2R,3R)-5-[(2-fluorophenylthio) methyl] oxolane-3,4-diol)

FFA: Free Fatty Acids

TG: Triglycerides

MAP: Mean Arterial Pressure

PIA: N^6-(L-2-Phenylisopropyl)adenosine

CPA: N^6-Cyclopentyladenosine

DPCPX: 1,3-Dipropyl-8-cyclopentylxanthine

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ABSTRACT

Elevated lipolysis and circulating free fatty acid (FFA) levels have been linked to the pathogenesis of insulin resistance. A_1 adenosine receptor agonists are potent inhibitors of lipolysis. Several A_1 agonists have been tested as potential anti-lipolytic agents; however, their effect on the cardiovascular system remains a potential problem for development of these agents as drugs. In the present study we report that CVT-3619, a novel partial A_1 receptor agonist, significantly reduces circulating FFA levels without any effect on heart rate and blood pressure in awake rats. Rats were implanted with indwelling arterial and venous cannulas to obtain serial blood samples, record arterial pressure, and administer drug. CVT-3619 decreased FFA levels in a dose-dependent manner at doses from 1 up to 10 mg/kg. The FFA lowering effect was blocked by A_1 receptor antagonist, DPCPX. Triglyceride (TG) levels were also significantly reduced by CVT-3619 treatment in the absence and presence of Triton. Tachyphylaxis of the anti-lipolytic effect of CVT-3619 (1 mg/kg, iv bolus) was not observed with three consecutive treatments. An acute reduction of FFA by CVT-3619 was not followed by a rebound increase of FFA as seen with nicotinic acid. The potency of insulin to decrease lipolysis was increased 4-fold ($p<0.01$) in the presence of CVT-3619 (0.5 mg/kg). In summary, CVT-3619 is an orally bioavailable A_1 agonist which lowers circulating FFA and TG levels by inhibiting lipolysis. CVT-3619 has anti-lipolytic effects at doses that do not elicit cardiovascular effects.

INTRODUCTION

There is considerable experimental and clinical evidence that elevated levels of circulating free fatty acids (FFA) play an important role in the pathogenesis of insulin resistance and diabetes (Chen et al., 1987;Reaven, 1995;Roden et al., 1996;Boden, 2001;Bays et al., 2004). Increases of adipose tissue mass and adipocyte cell size are associated with elevated blood FFA content. Enlarged adipocytes become resistant to the anti-lipolytic actions of insulin and release increased amounts of FFA into the circulation. Consequently, non-adipose tissues become exposed to elevated plasma FFA levels (Reaven, 1995;Boden, 2001;Bays et al., 2004). This results in increased deposition of triglycerides in peripheral tissues such as skeletal muscle, liver, pancreas and heart, rendering these organs resistant to the actions of insulin (Sako and Grill, 1990;Roden et al., 1996;Itani et al., 2002;Boden et al., 2005). Therefore, because FFA are central to the development of insulin resistance and lipid abnormalities of diabetes, reduction in elevated FFA levels is a goal in the treatment of insulin resistance and type 2 diabetes.

Despite overwhelming evidence of a role of elevated FFA in insulin resistance and diabetes, very few inhibitors of lipolysis are available for either experimental or clinical use. Nicotinic acid and its analogue acipimox are the only well-characterized anti-lipolytic agents that are currently used for treatment of dyslipidemia (Carlson, 2005;Vega et al., 2005). Their therapeutic usefulness is limited because the initial decrease in plasma FFA levels is followed by a rebound that transiently increases FFA and insulin resistance (Poynten et al., 2003). In addition, nicotinic acid has an unfavorable side-effect profile and is contra-indicated for the treatment of diabetic

patients (McKenny et al., 1994;Grundy et al., 2002;Poynten et al., 2003). Thus, there is a need for novel anti-lipolytic agents and better understanding of their potential usefulness in the treatment of insulin resistance and diabetes.

A_1 adenosine receptor agonists are well recognized anti-lipolytic agents due to their effect of reducing the formation and release of FFA from adipose tissue (Hoffman et al., 1986b;Gardner et al., 1994;Fraser et al., 2003;Dhalla et al., 2003;Schoelch et al., 2004;Fatholahi et al., 2006). A_1 agonists reduce lipolysis (breakdown of TG to FFA) in adipose tissue by inhibiting adenylyl cyclase activity and cAMP formation (Fain et al., 1972;Schwabe et al., 1974). The use and potential benefits of A_1 agonists to reduce lipolysis have been limited by the concurrent cardiovascular effects of this class of agents. The cardiac effects mediated by A_1 receptors include slowing of heart rate and AV nodal conduction, and depression of atrial contractility (Belardinelli et al., 1989). However, due to a greater receptor reserve in adipose tissue as compared to cardiac tissue (Wu et al., 2001;Liang et al., 2002), significant anti-lipolytic effects of A_1 agonists have been reported at doses that have either minimal or no cardiac effects (Gardner et al., 1994;Fraser et al., 2003). These findings suggest that it is possible to achieve organ selectivity for A_1 receptor mediated responses. In this regard, partial agonists of the A_1 receptor may be useful to minimize unwanted cardiac effects, as they elicit only sub-maximal responses in the heart even at high doses/concentrations (van Schaick et al., 1998;Wu et al., 2001).

CVT-3619, a derivative of adenosine, is a selective, partial agonist for the A_1 adenosine receptor that has been shown to inhibit lipolysis in isolated rat adipocytes at

concentrations that do not have significant effects in isolated heart (Fatholahi et al., 2006). The present study was undertaken to characterize the anti-lipolytic and cardiovascular properties of CVT-3619 *in vivo*. The data show that CVT-3619 lowers plasma FFA and triglyceride levels in a dose-dependent manner without significant cardiovascular effects and also increases the potency of insulin as an anti-lipolytic hormone**.**

MATERIALS AND METHODS

Animals: All experimental procedures were performed under a protocol approved by the Institutional Animal Care and Use Committee of CV Therapeutics, Inc., and in accordance with the recommendations set forth in the Guide for the Care and Use of Laboratory Animals published by the National Research Council. Male Sprague-Dawley rats (300-325 gm) with either one or two indwelling catheters (carotid and jugular) were purchased from Charles River Laboratories (Wilmington, MA). Animals were housed 1 per cage in a room maintained on a 12h light/dark cycle (light on 06.00-18.00 h) under constant temperature (22-25° C) with *ad libitum* access to food and water.

Experimental Protocol: The anti-lipolytic effects of CVT-3619 were studied in awake rats. Animals were fasted overnight before experimental use. On the day of the experiment, animals were put in metabolic cages and left undisturbed to acclimate to the environment for 1-2 hrs. An infusion set (21G x ¾", 0.8 x 19mm U.T.W., 3 ½", 9cm tubing, volume 0.15ml) was connected to the arterial catheter for blood sampling. A 1% sodium citrate saline solution was used to flush the lines. A pre-treatment blood sample was obtained from each animal to determine baseline values for FFA and TG. CVT-3619 was given via oral gavage, sc injection, iv injection, or ip injection, as described, for each different series of experiments. Blood samples were collected into serum separator tubes (Becton Dickinson, Franklin Lakes, NJ) at pre-determined times. Blood was allowed to clot, and then centrifuged at 8000 rpm for 5 min at 4ºC. The serum was stored at -80ºC and was thawed at 4ºC for determinations of FFA and TG contents.

Cardiovascular Measurements: The effects of CVT-3619 on heart rate and blood pressure were determined in a separate group of animals as heart rate is very easily affected in the un-anesthetized animal by animal handling and blood sampling. Rats were instrumented with radiotelemetered transmitters (Data Sciences) at least 3 weeks prior to experimentation. The ECG, blood pressure and temperature were recorded and heart rate calculated using a Dataquest ART Gold system (Version 2.2; Data Sciences Intl). The system consisted of a transmitter, i.e., biopotential sensor (Model TL11M2-C50-PXT), receivers (Model RPC-1), a consolidation matrix (BCM 100), a personal computer (Compaq DeskPro Series 3574) and Dataquest 4 software. Heart rate, blood pressure and temperature were measured at 5-minute intervals. Each recording lasted 10 seconds and all cardiac cycles within this period were averaged.

Chemicals and Reagents: CVT-3619 (2-{6-[((1R,2R)-2-hydroxycyclopentyl)amino] purin-9-yl}(4S,5S,2R,3R)-5-[(2-fluorophenylthio)methyl]oxolane-3,4-diol) was synthesized by the Department of Medicinal and Bio-Organic chemistry of CV Therapeutics, Inc[1]. Sodium citrate, DPCPX (1,3-Dipropyl-8-cyclopentylxanthine), CPA (N^6-Cyclopentyladenosine) and Triton WR1339 were purchased from Sigma (St. Louis, MO). Nicotinic acid and PEG 400 were purchased from VWR (by EMD Chemicals). DPCPX was dissolved in 35% ethanol/ 65% water. CPA was dissolved in deionized water. Triton WR 1339 was diluted in warm saline (~37ºC) with frequent vortexing. Nicotinic acid was dissolved in saline. CVT-3619 was dissolved in PEG 400 by sonicating for 20 min and then diluted with distilled water to make a 20% PEG drug

solution. FFA and TGs were measured using commercial kits from Wako Chemicals, (Richmond, VA). Glucose and Insulin were measured using commercial kits from Thermo Electron Corporation (Waltham, MA) and Crystal Chem (Downers Grove, IL), respectively.

Data Analysis: All data are reported as mean ± SEM. Statistical analysis of data from experiments with 2 treatment groups was performed using the unpaired Student's *t*-test. Two way analysis of variance followed by Bonferroni's test was used for multiple comparisons. Differences among treatment groups were considered to be significant when the probability of their occurrence by chance alone was < 0.05.

RESULTS

Effect of CVT-3619 on Plasma Free Fatty Acid and Triglyceride Levels: CVT-3619 lowered FFA levels in a dose-dependent manner in normal, overnight-fasted awake rats. The time course of the effect of CVT-3619 on circulating serum FFA levels is shown in Figure 1A. There was a small increase in FFA levels in the vehicle group at 10 min after the vehicle gavage. This response is likely due to an increase in lipolysis caused by the increase in sympathetic tone associated with the handling of awake animals. CVT-3619 at a dose of 2.5 mg/kg lowered FFA levels from 0.7 ± 0.05 to 0.5 ± 0.03 mM, a 31% decrease below baseline levels ($p<0.05$). CVT-3619 lowered FFA levels by 47% to 0.4 ± 0.03 from 0.8± 0.04 mM at a dose of 5 mg/kg dose ($p<0.01$). A 10 mg/kg dose caused a 57% decrease in FFA levels (from 0.68 ± .04 to 0.29 ± 0.02 mM, $p<0.001$). The duration of the effect of CVT-3619 to suppress lipolysis was also dose-dependent (figure 1A).

To determine whether the A_1 receptor subtype was responsible for mediating the FFA lowering effect of CVT-3619, rats were pretreated with DPCPX, an A_1 receptor antagonist, 10 min prior to administration of CVT-3619 (figure 1B). DPCPX (1 mg/kg) itself caused a small increase in FFA levels. This is expected as DPCPX is not a neutral antagonist but instead an inverse agonist (Shryock et al., 1998). As clearly shown in figure 1B, pre-treatment with DPCPX completely prevented the decrease in FFA caused by CVT-3619 (5 mg/kg).

CVT-3619 reduced serum triglyceride levels in a dose-dependent manner. The effect of three doses of CVT-3619 on serum triglycerides at 60 minutes post-treatment is shown in Figure 2. TG levels were significantly decreased ($p<0.05$) from 54 ± 4 to 35 ± 4 mg/dl at a dose of 2.5 mg/kg of CVT-3619, representing a 36% decrease. Doses of 5 and 10 mg/kg of CVT-3619, caused a 41% (32 ± 4 mg/dl, $p<0.01$) and 58% (23 ± 1 mg/dl, $p<0.01$) reduction in TG levels, respectively, compared to vehicle-treated rats.

Effect of CVT-3619 on Triglyceride Production: To further investigate the mechanism of the decrease in TG levels by CVT-3619, total TG production was measured in normal rats. TG production was estimated by comparing the accumulation of TG in the plasma after an injection of Triton WR 1339 (Triton, 600 mg/kg) in the absence and presence of CVT-3619 (Figure 3). Treatment of rats with Triton caused a time-dependent increase in serum TG in both vehicle- and CVT-3619-treated rats. The increase in serum TG caused by Triton was significantly less in CVT-3619-treated animals as compared to the vehicle-treated animals at 180 minutes post-treatment ($p<0.01$). TG accumulation as determined from the slope of the line (linear regression of the data) was also significantly less ($p< 0.001$) in rats treated with CVT-3619 (5.6 ± 0.12 mg/dl/min) as compared to vehicle-treated rats (3.8 ± 0.17 mg/dl/min).

Lack of Tachyphylaxis to Repeated Treatment with CVT-3619: The decrease in FFA levels caused by CVT-3619 was highly reproducible and did not undergo acute tachyphylaxis. As shown in Figure 4, three repeated iv injections of CVT-3619 (1

mg/kg) to rats caused similar decreases in FFA levels to 0.35 ±0.04, 0.35 ± 0.03 and 0.38 ± 0.03 mM, respectively, from a baseline value of 0.88 ± 0.02 mM. The time-course of the decreases in plasma FFA levels caused by the three consecutive injections of CVT-3619 was similar.

No Rebound with CVT-3619: The anti-lipolytic effect of CVT-3619 was compared to that of nicotinic acid in overnight-fasted awake rats. CVT-3619 and nicotinic acid lowered FFA levels to 0.36 ± 0.05 from 0.79 ± 0.04 mM ($p< 0.001$) and 0.35 ± 0.01 from 0.85 ± 0.09 nM ($p<0.001$), respectively (Figure 5). CVT-3619 (1 mg/kg, iv bolus) caused a maximal 54 ± 5% decrease in FFA levels which was comparable to that caused by nicotinic acid (57 ± 5%) given at a dose of 10 mg/kg iv bolus. The rebound increase of FFA levels seen with nicotinic acid was not observed with CVT-3619.

Effect of CVT-3619 and Insulin on FFA Levels: The effect of insulin (0.005-1U/kg) to reduce serum FFA was determined in the absence and presence of a single dose (0.5 mg/kg) of CVT-3619 (Figure 6). Baseline FFA levels in vehicle and CVT-3619 groups were 0.84 ± 0.01 and 0.92 ± 0.02 mM, respectively. CVT-3619 alone (0.5 mg/kg) caused an 18% decrease in FFA levels. As expected, insulin lowered FFA levels by up to 67 ± 1 % in a dose-dependent manner. The doses of insulin that caused 50% decrease (ED_{50}) in FFA levels in the absence and presence of CVT-3619 were 0.4 and 0.1 U/kg, respectively. Thus, in the presence of CVT-3619, there was a 4-fold leftward shift of the insulin dose-response to lower FFA suggesting that CVT-3619 increases insulin sensitivity in adipose tissue.

Cardiovascular Effects of CVT-3619: The effects of CVT-3619 on heart rate and blood pressure were determined in awake rats by telemetry and the data are shown in Figure 7. CVT-3619 at doses of 1 and 5 mg/kg did not have a significant effect on heart rate but caused a small decrease (13 ± 1% calculated as area under the curve) in heart rate at a dose of 25 mg/kg (Figure 7A). Increasing the dose of CVT-3619 to 50 mg/kg caused no further decrease in heart rate (data not shown). CVT-3619 did not have any significant effect on blood pressure at the doses used (Figure 7B).

Comparison between a Full A_1 Agonist CPA and the partial A_1 Agonist CVT-3619: The antilipolytic and hemodynamic effects of CVT-3619 were also compared with that of CPA, a full agonist of the A_1 adenosine receptor. The results are shown in Figure 8. CPA caused a 56% decrease from baseline in FFA levels at a dose of 20 μg/kg given via an ip injection (Figure 8A). At the same dose CPA caused significant bradycardia (from 387 to 187 bpm) which lasted for almost 30 minutes post-treatment (Figure 8B). In contrast, CVT-3619 caused a similar decrease in FFA levels at a dose of 10 mg/kg (figure 8A) but had no significant effect on heart rate (Figure 8B). A small transient increase in heart rate seen with CVT-3619 is likely due to increase in sympathetic tone caused by the handling of the rats and was also noted with vehicle treatment as shown in figure 7.

Discussion

High circulating FFA levels contribute to the development of insulin resistance and are considered a risk factor for type II diabetes and metabolic syndrome (Reaven, 1995). Adipose tissue lipolysis, which is highly regulated by insulin under normal conditions, is the major determinant of plasma FFA concentrations (Arner, 2005). However, the anti-lipolytic effect of insulin is impaired in insulin resistant states, leading to an increased rate of lipolysis and high circulating FFA levels. Thus, anti-lipolytic agents that can normalize the rate of lipolysis in insulin-resistant states should be clinically useful.

Recently we have shown that CVT-3619 is a selective and partial A_1 adenosine receptor agonist, with anti-lipolytic activity (Fatholahi et al., 2006). CVT-3619 inhibited cAMP accumulation and FFA release from rat adipocytes, and had minimal effects on cardiac function in isolated heart preparation (Fatholahi et al., 2006). The present study investigated the *in vivo* metabolic and cardiovascular effects of CVT-3619 in awake rats. The results show that CVT-3619 lowers FFA and TG in a dose-dependent manner. The FFA-lowering effect does not undergo tachyphylaxis and is not associated with a rebound. The anti-lipolytic effects occur at doses that have no significant cardiovascular effects. The anti-lipolytic effects of CVT-3619 were also compared to that of nicotinic acid, a potent and clinically used anti-lipolytic agent. Last but importantly, CVT-3619 increased the potency of insulin to reduce plasma FFA concentrations.

CVT-3619 lowered circulating FFA levels in a dose-dependent manner. This data is consistent with previous reports showing that other A_1 agonists decrease FFA levels (Hoffman et al., 1986b;Gardner et al., 1994;Fraser et al., 2003;Schoelch et al., 2004). The FFA lowering effect of CVT-3619 was completely antagonized by pre-treatment with an A_1 antagonist, DPCPX, confirming that these effects are mediated via A_1 receptors. CVT-3619 also caused a significant decrease in TG levels. The decrease in TG secretion is likely due to decreased substrate (FFA) availability in the liver, as has been shown previously (Hoffman et al., 1986b;Gardner et al., 1994). Limiting the supply of FFA to the liver decreases the output of triacylglcerol (VLDL) and ketone bodies thus producing both hypotriglyceridemic and anti-ketotic effects (Kovoor et al., 1998). Although we did not measure VLDL production, the decrease in TG production by CVT-3619 is also expected to result in decreased VLDL production as previously shown using R-phenylisopropyladenosine (R-PIA) (Hoffman et al., 1986b).

Tachyphylaxis and receptor desensitization are potential problems when considering a receptor agonist as a drug for long-term use. It has been shown that A_1 receptors undergo agonist-induced long-term desensitization but are not subject to rapid acute desensitization (Gao et al., 1999). The anti-lipolytic effects of CVT-3619 were well-maintained over three consecutive administrations. The magnitude and the duration of the FFA-lowering effect of CVT-3619 were similar for all three injections suggesting that the effect of this agonist does not undergo tachyphylaxis. Desensitization of the anti-lipolytic effect of A_1 receptors has been shown to occur with prolonged and continuous exposure to high concentrations of an A_1 agonist, R-PIA

(Hoffman et al., 1986a). R-PIA is a full agonist and thus more likely to cause desensitization. In contrast to R-PIA, CVT-3619 is a partial A_1 receptor agonist. Partial agonists of GPCRs have been suggested to cause less receptor desensitization than full agonists (Vachon et al., 1987;Kovoor et al., 1998). Whether the anti-lipolytic effect of CVT-3619 is sustained over long-term use (months) remains to be determined; however, we do have preliminary data showing that the anti-lipolytic effects of CVT-3619 given twice daily are well-maintained up to 6 weeks of treatment (unpublished data).

The FFA-lowering effect of CVT-3619 was comparable to that of nicotinic acid (10 mg/kg). Nicotinic acid, a ligand for the HM74A receptor, is a short-acting, potent inhibitor of lipolysis (Carlson and Oro, 1962;Tunaru et al., 2003). Its use is limited by side effects such as flushing and a post-treatment rebound increase in FFA (McKenny et al., 1994). The suppression of lipolysis by nicotinic acid is followed by a rebound in FFA release, such that the levels of FFA rise above the baseline upon washout of the effect (Pereira, 1967;Blackard and Heidingsfelder, 1969). The rebound has been suggested to be responsible for the paradoxical decrease in insulin sensitivity observed when using large doses of nicotinic acid (Kelly et al., 2000;Poynten et al., 2003). The mechanism of FFA rebound with nicotinic acid remains unknown. It has been suggested that the magnitude of rebound is dependent upon the magnitude of decrease in FFA and a significant correlation between FFA lowering and rebound has been shown for nicotinic acid (Blackard and Heidingsfelder, 1969;Schwabe et al., 1974). Rebound increase in plasma FFA levels was not observed with CVT-3619, even though

FFA concentrations were decreased by similar extent by both CVT-3619 and nicotinic acid, i.e. 54% and 57% from baseline, respectively. It has been shown that the FFA rebound still exists with an extended-release nicotinic acid formulation (Vega et al., 2005), which suggests that the rebound phenomenon may be unique to nicotinic acid and may not apply to other anti-lipolytic agents.

The anti-lipolytic actions of adenosine that are mediated by A_1 receptors have been known for many years (Hoffman et al., 1986b;Gardner et al., 1994;Fraser et al., 2003;Schoelch et al., 2004). The metabolic responses after acute administration of many A_1 agonists have been reported previously; however, no compound has been developed and approved for clinical use thus far. A possible reason for this is lack of separation between the cardiac (and perhaps CNS) and the anti-lipolytic effects (Dhalla et al., 2003). It is possible, however, to achieve functional selectivity using partial agonists as previously described (van Schaick et al., 1998;Wu et al., 2001). The position of the dose or concentration response relationship for the anti-lipolytic and cardiovascular effects of CVT-3619 are further apart than for full A_1 receptor agonists such as CPA (Figure 8). CPA caused marked bradycardia at a dose (i.e. 20 μg/kg) that caused a similar decrease in FFA levels as that observed with CVT-3619 at 10 mg/kg which had no effect on heart rate. Although some degree of functional selectivity can also be achieved with full agonists (Fraser et al., 2003) the difference between the effective dose to lower FFA and to depress cardiac function is greater for partial than full agonists making them much safer drugs. Functional selectivity of CVT-3619 to decrease lipolysis and to lower FFA levels relative to heart rate was greater than 25-fold (compare

figure 1 and figure 7). This differential response to CVT-3619 results from the much higher sensitivity of adipose tissue (as compared to cardiac tissue) to adenosine analogues. The differential sensitivity to A_1 agonists has been explained on the basis of the differences in the receptor reserve in the two tissues (Liang et al., 2002;Fatholahi et al., 2006). The possibility of the existence of different receptors in the heart and adipose tissue has previously been ruled out (Tatsis-Kotsidis and Erlanger, 1999;Fatholahi et al., 2006).

Adenosine has been shown to modulate insulin actions and insulin sensitivity in muscle and adipose tissue (Budohoski et al., 1984;Rolband et al., 1990). In adipocytes, the increase in insulin sensitivity by adenosine was suggested to be mediated by A_1 receptors. Phenylisopropyladenosine (PIA), an A_1 adenosine receptor agonist, potentiated the insulin-induced activation of PI3 kinase, a second messenger for insulin actions, in rat adipocytes (Takasuga et al., 1999). Our data show that in the presence of CVT-3619 the EC_{50} for insulin to inhibit lipolysis in-vivo is decreased 4-fold, suggesting that CVT-3619 increases insulin sensitivity in adipose tissue. This potentiation of the FFA lowering effect occurs at a much lower dose of CVT-3169 (0.5 mg/kg) than those used for investigating the anti-lipolytic effects of CVT-3169 alone (1.0 mg/kg and higher). Thus, CVT-3619 could be useful in insulin resistant states where anti-lipolytic effect of insulin is impaired and the rate of lipolysis is increased leading to high circulating levels of FFA.

In conclusion, data in the present study show that CVT-3619, an A_1 adenosine receptor partial agonist, is an effective anti-lipolytic agent that lowers circulating FFA and TG levels, and improves insulin sensitivity in adipose tissue. The anti-lipolytic effect of CVT-3619 is not associated with a rebound increase FFA. The FFA-lowering effects occur at doses that have no effect on heart rate and blood pressure. The pharmacological properties of CVT-3619 suggest that this compound may have therapeutic utility in metabolic and cardiovascular disorders in which FFA levels are increased.

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Footnote on Page 8

[1] For complete synthesis process for CVT-3619 refer to the US patent Pub. No. US 2006-0009417 and US2006-0052330.

Legends for Figures

Figure 1: Anti-lipolytic effect of the partial A_1 agonist CVT-3619. Shown is (A) the time-course of the effect of various doses of CVT-3619 on circulating free fatty acids (FFA) in awake rats. Three doses (2.5, 5 and 10 mg/kg) of CVT-3619 were administered via an oral gavage after an overnight fast. Panel B shows the antagonism of the FFA lowering effect of CVT-3619 by A_1 receptor antagonist DPCPX. DPCPX (1 mg/kg, ip) was given 10 minutes prior to CVT-3619 (5 mg/kg) administration. Each symbol represents the mean ± SEM of the FFA levels from the number of rats indicated in parenthesis for each group. *) $p<0.05$, **) $p<0.01$, ***) $p< 0.001$, indicating values that are significantly different from vehicle treated group.

Figure 2: Lipid lowering effect of CVT-3619. Shown is the effect of various doses of CVT-3619 on serum triglycerides (TG) in awake rats at 60 min after treatment. Three doses (2.5, 5 and 10 mg/kg) of CVT-3619 were administered via an oral gavage after an overnight fast. Data are presented as mean ± SEM of the TG levels from the number of rats indicated in parenthesis for each group. *) $p< 0.05$, **) $p<0.01$ indicates values that are significantly different from vehicle (0) treated.

Figure 3: Time-dependent increase of serum triglyceride (TG) caused by Triton WR 1339 in the absence and presence of CVT-3619. After 4 hrs fast, rats received either vehicle or CVT-3619 (5 mg/kg) via a sc injection. After 5 min, Triton (400 mg/kg) was given as a slow intravenous bolus. Data are presented as mean ± SEM of values from 7-8 animals. Slope of the lines (determined by linear regression analysis) was 5.6 ± 0.1

and 3.8 ± 0.2 for vehicle and CVT-3619 groups, respectively. Data were analyzed using 2 way ANOVA followed by Bonferroni's post hoc test.

Figure 4: Lack of acute desensitization (tachyphylaxis) of the FFA lowering effect of CVT-3619. Shown is effect of three consecutive injections of CVT-3619 on serum FFA levels in awake rats. Animals were fasted overnight and CVT-3619 was given (iv bolus) at a dose of 1 mg/kg. Arrows indicate the times of CVT-3619 administrations. Data are presented as mean ± SEM values of FFA from nine controls (vehicle treated) and five CVT-3619 treated rats.

Figure 5: Nicotinic acid, but not CVT-3619, causes an increase in FFA following an initial decrease. Shown is the time-course of the effects of CVT-3619 and nicotinic acid on serum FFA in awake rats. Animals were fasted overnight and were treated with vehicle, CVT-3619 or nicotinic acid via an iv bolus injection. Data are presented as mean ± SEM of the FFA level from four to eight rats in different groups. *) $p<0.001$ indicates significantly different from vehicle at the same time point.

Figure 6: CVT-3619 potentiates the effect of insulin to reduce FFA levels. Shown are the dose response curves for the effect of insulin to reduce FFA obtained in the absence and presence of CVT-3619 (0.5 mg/kg) in awake rats. Both Insulin and CVT-3619 were given via ip injection. Each data point is the mean ± SEM of the maximal (peak effect) percent decrease in FFA levels from baseline from three to five rats. The doses of insulin that cause 50% decrease (ED_{50}) in FFA levels in the absence and

presence of CVT-3619 were 0.4 (0.3916-0.4208, 95% CI) and 0.1 (0.0935-0.133) U/kg, respectively.

Figure 7: Time-course of the effects of CVT-3619 on (A) heart rate and (B) mean arterial pressure (MAP) in awake rats as measured by telemetry. CVT-3619 was given at various doses (1, 5 and 25 mg/kg by an oral gavage) at time 0. Each data point is the mean of individual values from the number of experiments indicated in parenthesis. The error bars have been omitted for clarity. Note that the initial transient (10 min) increase in heart rate subsequent to the injection of vehicle or CVT-3619 is due to the stress caused by the handling of the animals.

Figure 8: Comparison of the (A) anti-lipolytic effect and (B) cardiac effects of CVT-3619 to that of full agonist CPA. For FFA measurements, animals were fasted overnight and were treated with CVT-3619 (10 mg/kg, po) or CPA (20 μg/kg, ip). Effect of CVT-3619 and CPA on heart rate was determined by telemetry in awake rats. Data are presented as mean ± SEM from the number of animals indicated in the parenthesis after each group.

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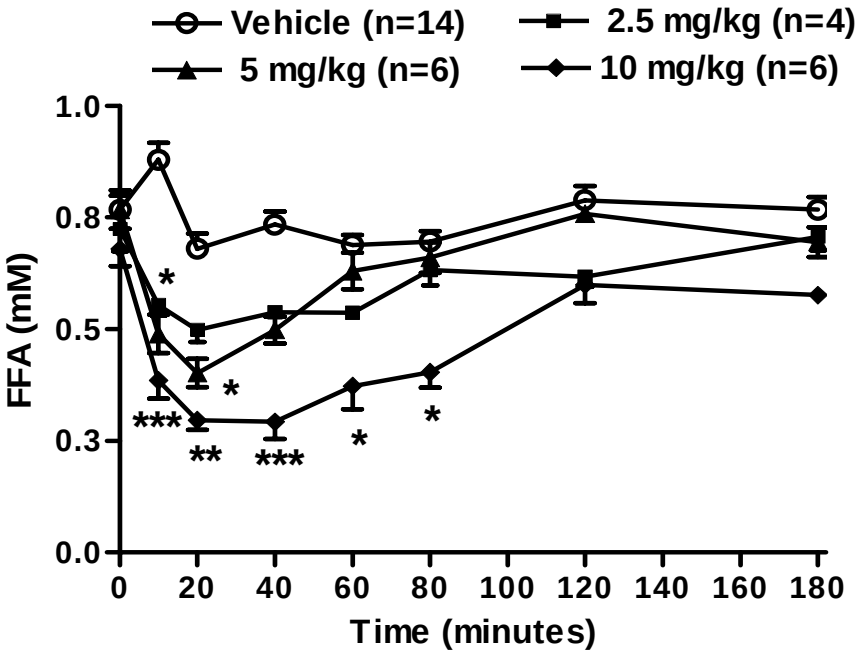

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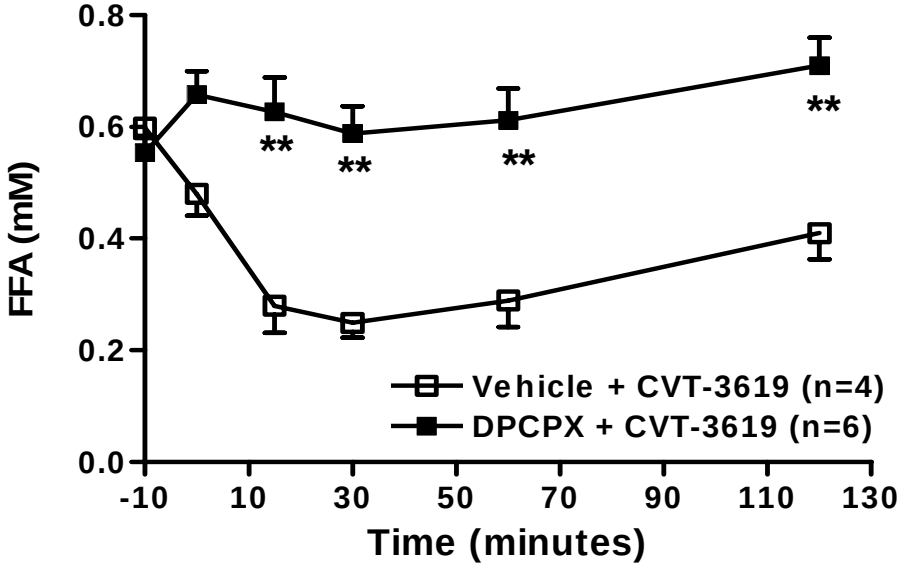

Figure 1

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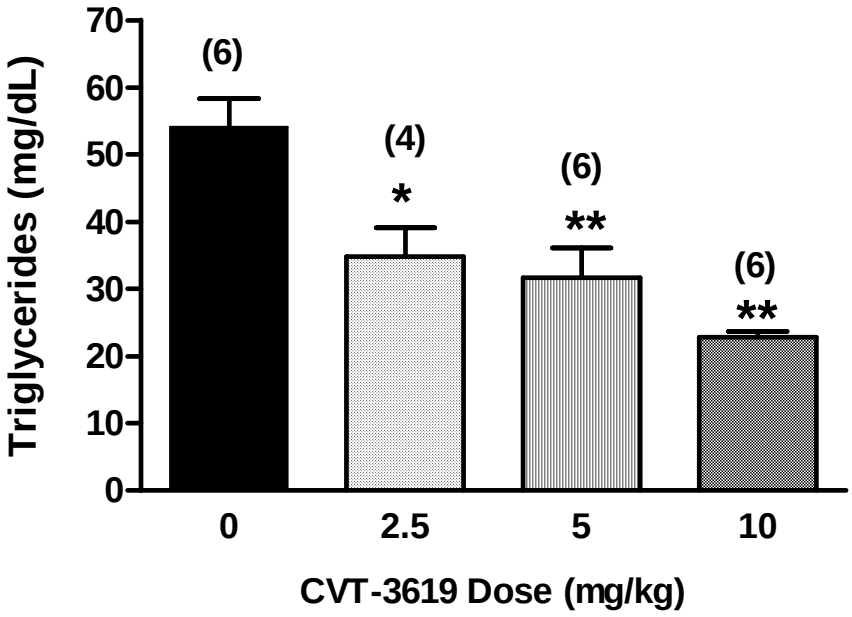

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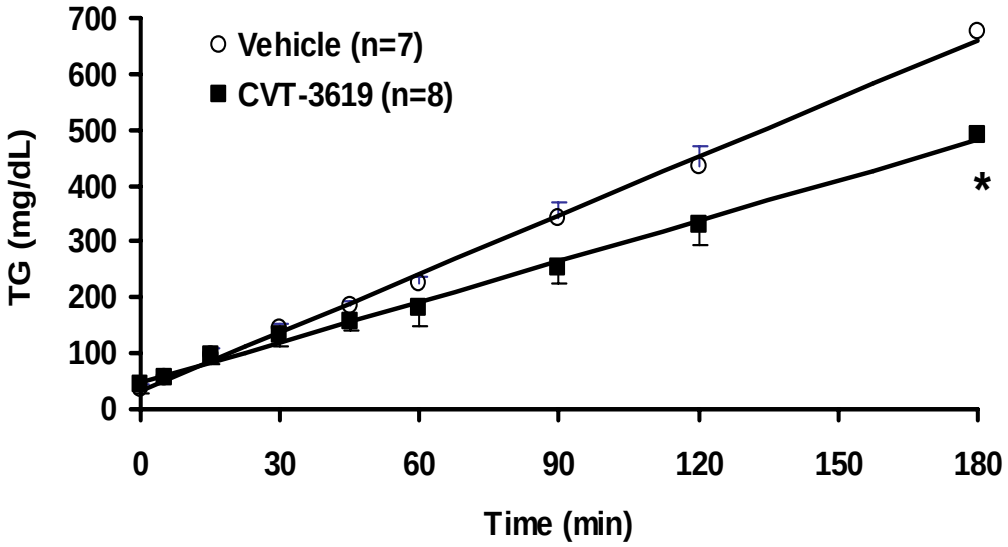

Figure 3

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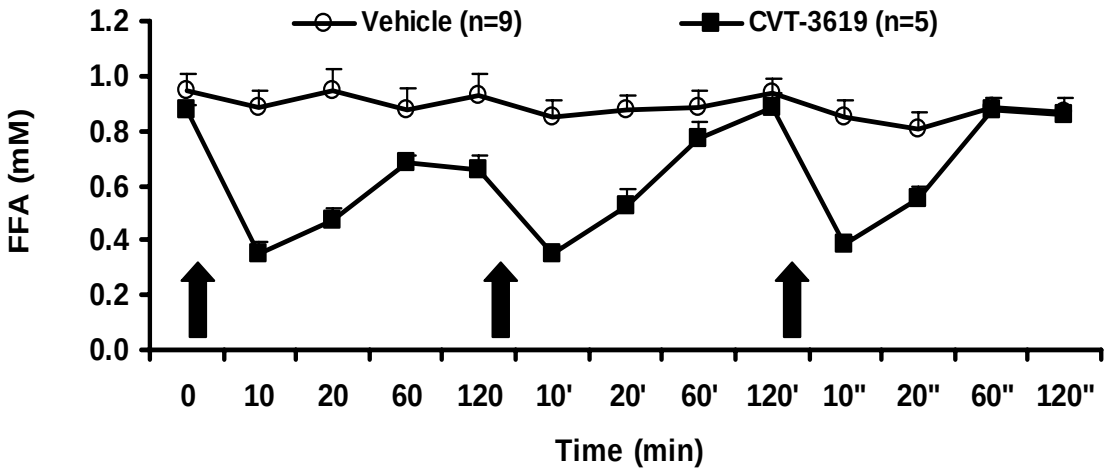

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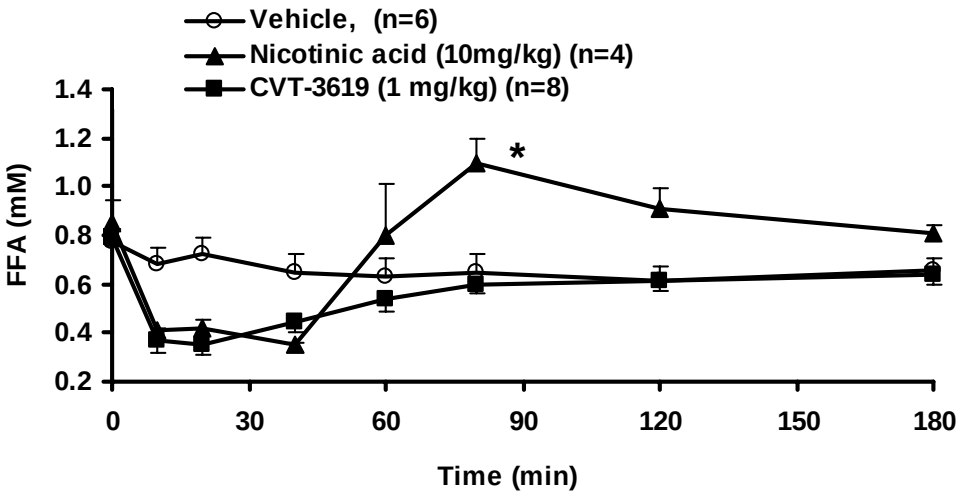

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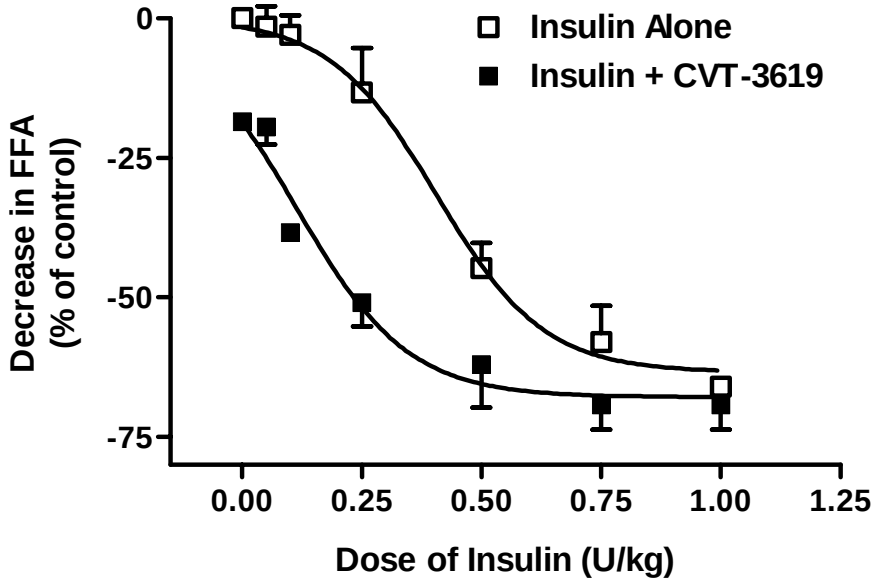

Figure 6

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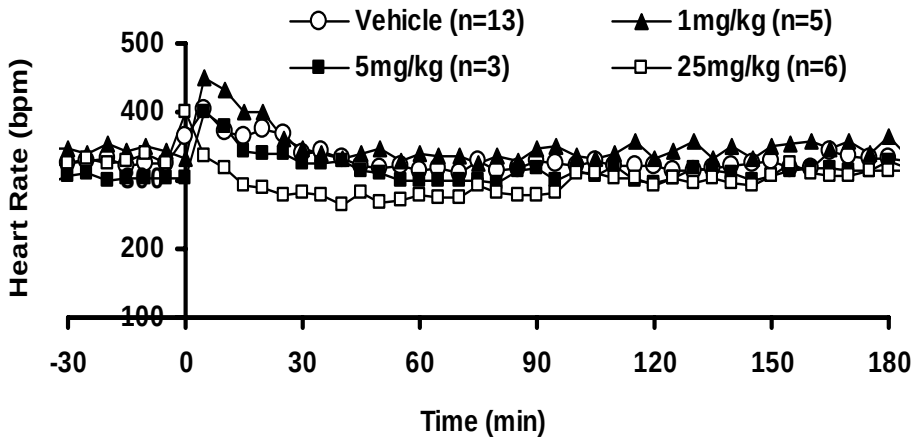

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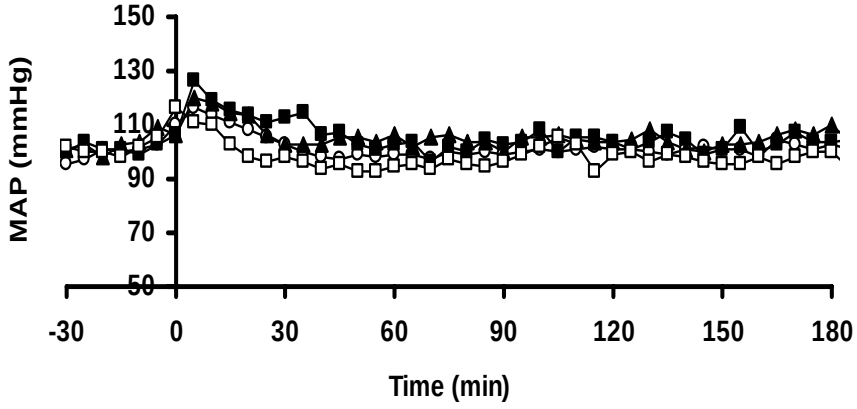

Figure 7

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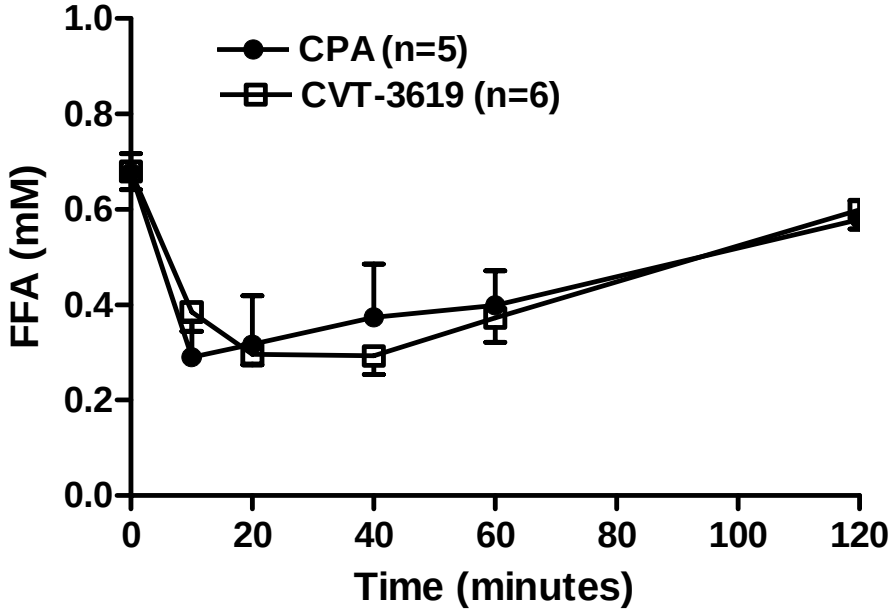

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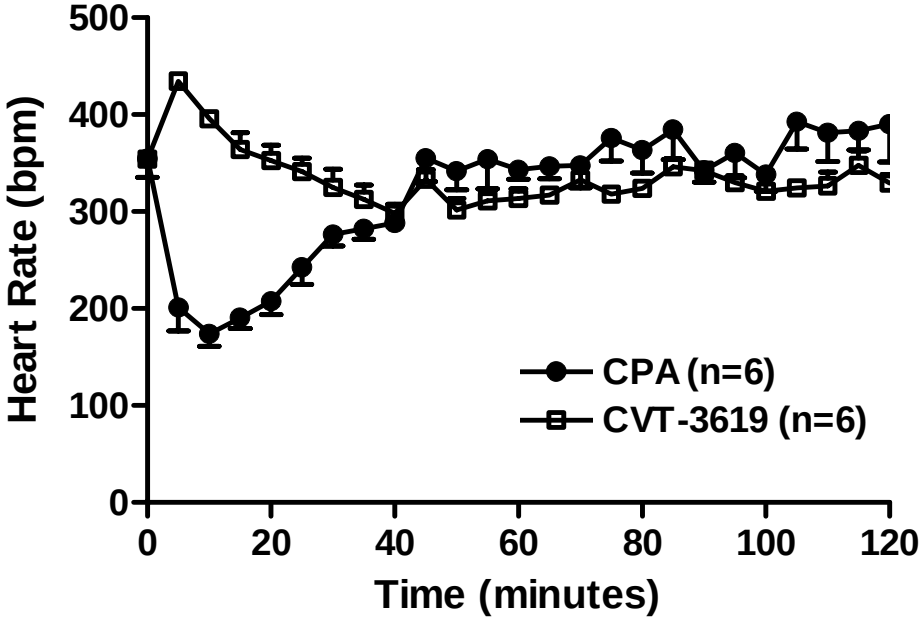

Figure 8