N-[3-(R)-Tetrahydrofuranyl]-6-aminopurine Riboside, an A₁ Adenosine Receptor Agonist, Antagonizes Catecholamine-Induced Lipolysis without Cardiovascular Effects in Awake Rats

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ABSTRACT

Elevated serum nonesterified free fatty acid (NEFA) concentrations are detrimental to both the mechanical and electrical function of the heart. A₁ adenosine receptor agonists are potent and efficacious inhibitors of lipolysis; however, their cardiovascular effects have limited their use to lower serum NEFA. Our objective was to determine whether the antilipolytic effect of N-[3-(R)-tetrahydrofuranyl]-6-aminopurine riboside (CVT-510), an A₁ agonist, could be distinguished from its bradycardia effect and demonstrated in rats with normal or elevated serum NEFA. Rats were instrumented with telemetry transmitters for continuous recording of heart rate, and catheterized, for delivery of drugs and blood sampling. CVT-510 caused a rapid and sustained dose-dependent decrease in NEFA at doses that did not cause bradycardia (2, 5, and 20 µg/kg). Significant bradycardia was observed at 50 µg/kg. Norepinephrine (NE) increased NEFA from 0.5 ± 0.01 to 0.9 ± 0.2 mM and this effect lasted for 2 h. CVT-510 (10 µg/kg) given at 40 min postinjection of NE reversed the rise in NEFA (69% reduction). When CVT-510 (20 µg/kg) was given 15 min before a 30-min long infusion of NE, the lipolytic response to NE was prevented. To mimic the antilipolytic effect of CVT-510 in awake rats, hearts were perfused with palmitate at concentrations similar to those observed in the in vivo studies (0.8 and 0.2 mM), which decreased myocardial oxygen consumption (MVO₂) by 11%. Thus, CVT-510 at doses ≥5-fold lower than those that slow heart rate caused a marked and sustained lowering of normal or elevated NEFA, that when mimicked in vitro decreased MVO₂ and would be expected to improve cardiac efficiency.

Fatty acids are the major substrate for ATP production in the heart (Neely and Morgan, 1974) and can account for as much as 70% of the ATP produced by normoxic cardiac myocytes. Therefore, under normal conditions, the oxidation of fatty acids is essential to maintain adequate energy production in the heart. However, under certain conditions, such as ischemia and hypoxia, elevated serum nonesterified free fatty acid (NEFA) concentrations have been shown to be detrimental to both the mechanical and the electrical functions of the heart (Opie et al., 1977; Kurien et al., 1971; Oliver, 2001b). The utilization of free fatty acids by the myocardium is regulated by their plasma concentration, the rate of β-oxidation and the functional demands on the heart. Thus, when concentrations of NEFA are high, the oxidation of fatty acids is also high (Neely and Morgan, 1974; Longnus et al., 2001). Although inhibition of β-oxidation of fatty acids is an established therapeutic target, an alternative approach would be the pharmacological reduction of free fatty acids and thus their utilization, with the objective to minimize their detrimental effects (Stanley et al., 1997; Oliver, 2001a,b).

Conditions whereby sympathetic tone is high (elevated catecholamines), such as during coronary artery bypass grafting (Kalman et al., 1995), acute myocardial ischemia (Oliver and Kurien, 1969; Maroko et al., 1971), and surgery (Hirvonen et al., 1978), are associated with elevated serum NEFA concentrations (Mjos et al., 1974; Lopaschuk et al., 1994), arrhythmias (Oliver and Kurien, 1969; Opie, 1988; Kalman et al., 1995), and left ventricular dysfunction (Henderson et al., 1970). Both arrhythmias and left ventricular dysfunction have been linked to elevated plasma NEFA concentrations (Henderson et al., 1970; Opie, 1988; Kalman et al., 1995) but a cause and effect relationship between the two remains tentative. Regardless, myocardial oxygen consump-
effects of NEFA was obtained from BIOMOL Research Laboratories (Plymouth Meeting, PA). Activation of $\alpha_1$ receptors by adenosine and adenosine analogs causes slowing of heart rate and AV nodal conduction, and depresses atrial contractility (Belardinelli et al., 1989; Wu et al., 2001). In addition to these direct effects, by inhibiting the activity of adenyl cyclase, $\alpha_1$ receptor activation counteracts the effects of $\beta$-adrenergic agonists (Dobson et al., 1987). $\alpha_1$ agonists have been shown to be potent antilipolytic agents (Strong et al., 1993; Merkel et al., 1995; van Schaick et al., 1998). Antilipolytic effect of $N$-[3-(R)-tetrahydrofuran-6-yl]adenosine 5'-riboside (CVT-510), a selective $\alpha_1$ adenosine receptor agonist (Snowdy et al., 1999), could be differentiated from its negative chronotropic effects, to determine whether CVT-510 could reverse a norepinephrine-induced stimulation of serum NEFA concentrations in awake rats and to identify a meaningful functional consequence in cardiac tissue that would provide evidence that the antilipolytic actions of CVT-510 could be beneficial to the heart.

Materials and Methods

Chemicals and Biological Reagents. CVT-510 was synthesized by the Department of Medicinal and Bio-Organic Chemistry (CV Therapeutics, Inc., Palo Alto, CA). Collagenase Type I was purchased from Worthington Biochemicals (Lakewood, NJ). The following were purchased from Sigma-Aldrich (St. Louis, MO): fatty acid-free bovine serum albumin (BSA), nicotinic acid, methylene adenosine 5'-diphosphate, $R$-(-)-$N^0$-[2-phenylisopropyl]adenosine ($R$-PIA), 8-cyclopentyl-1,3-dipropylxanthine (CPX), rolipram, isoproterenol, norepinephrine (NE) and $N^0$-cyclopentyltheophylline. Adenosine deaminase was obtained from Roche Diagnostics (Indianapolis, IN) and cilostamide was obtained from BIOMOL Research Laboratories (Plymouth Meeting, PA). The $\alpha_1$-adenosine receptor antagonist radioligand $[^3H]$CPX was purchased from PerkinElmer Life Sciences (Boston, MA). Stock solutions of adenosine receptor ligands, rolipram, and cilostamide were prepared in DMSO and stored at $-20^\circ$C. The maximum percentage of DMSO in each experiment was 0.1%. Isoproterenol stock solutions were prepared in 5 mM HCl, and solutions of methylene adenine 5'-diphosphate were prepared in saline.

Animals. Male Sprague-Dawley rats (380–420 g) were purchased from Simonsen Laboratories (Gilroy, CA). All animals received humane care according to the guidelines set forth in The Principles of Laboratory Animal Care formulated by the National Society for Medical Research and the Guide for the Care and Use of Laboratory Animals prepared by the Institute of Laboratory Animal Resources and published by the National Institute of Health (NIH Publication 85-23, revised 1996).

Isolation of Rat Epididymal Adipocytes. Adipocytes were isolated from the epididymal fat pads of rats as described previously (Rodbell, 1964). Briefly, rats were anesthetized using methoxyflurane and killed by exsanguination. Epididymal fat tissue was removed and placed into a modified Krebs (KRH) solution containing NaCl (100 mM), KCl (4.7 mM), CaCl$_2$ (2.5 mM), NaHCO$_3$ (3.6 mM), MgSO$_4$ (1.19 mM), KH$_2$PO$_4$ (1.18 mM), dextrose (5 mM), pyruvic acid (5 mM), ascorbic acid (1 mM), and HEPES (5 mM), pH 7.4. Visible blood vessels were dissected and excised, and the adipose tissue was minced. Minced tissue was digested with 25 ml of fresh KRH solution containing type I collagenase (1 mg/ml), fatty acid-free BSA (1% wt/vol) and nicotinamide acid (2 μM, to inhibit lipolysis) for 40 to 60 min at 37°C with continuous gentle shaking. The cell suspension was filtered through a nylon-mesh (210 μm) to remove undigested tissue fragments. The cell filtrate was washed three times using KRH solution containing 1% fatty acid-free BSA at 37°C. The final adipocyte suspension was either diluted in fresh KRH solution with 1% fatty acid-free BSA for use in cAMP experiments, or used to prepare membranes for radioligand binding assays.

cAMP Assays in Isolated Rat Adipocytes. Aliquots (100 μl, 45,000–90,000 cells) of the freshly prepared adipocyte cell suspension were placed into wells of 24-well cell culture plates containing 0.4 ml of KRH solution containing fatty acid-free BSA (1%), ascorbic acid (1 mM), rolipram (10 μM), cilostamide (1 μM), adenosine deaminase (2 U/ml), and appropriate $\alpha_1$ adenosine receptor agonist(s). An aliquot of 0.5 ml of KRH solution containing 60 nM isoproterenol was added to each well, and incubations proceeded for 4 min in an orbital shaker bath maintained at 37°C. Assays were terminated by the addition of 200 μl of 300 mM HCl to each well to lyse the cells. The concentration of cAMP in the cell lysate was determined using colorimetric direct cAMP kits (Assay Designs, Inc., Ann Arbor, MI).

Adipocyte Membrane Preparation and Competition Binding Assays. Freshly isolated adipocytes were added to a chilled solution containing sucrose (0.25 M), EDTA (1 mM), and Tris-HCl (10 mM, pH 7.4) and homogenized with 10 strokes using a motor-driven tissue grinder. The homogenate was cooled on ice and the fat layer was discarded. The homogenate was then centrifuged at 500g for 10 min at 4°C. The supernatant under the fat layer was removed, resuspended in fresh buffer, and homogenized a second time with six strokes using the tissue grinder. Cell membranes were collected by centrifugation of the homogenate at 15,000g for 15 min. The final membrane pellet was resuspended in a solution containing sucrose (0.25 M), phenylmethylsulfonyl fluoride (0.1 mM), leupeptin (5 μg/ml), aprotinin (5 μg/ml), adenosine deaminase (2 U/ml), and Tris-HCl (10 mM) buffer, pH 7.4. The membrane suspension was frozen and stored in liquid nitrogen. For competition binding assays, membrane suspensions were thawed and incubated for 2 h at room temperature in Tris-HCl (50 mM) buffer containing ADA (1 U/ml), guanosine 5'-($\beta$,γ-imido)triphosphate (100 μM), and $[^3H]CPX$ (1–3 nM) and progressively higher concentrations of the competing agent. At the end of incubation, free radioligand was separated from membrane-bound radioligand by filtration through GF/C glass fiber filters (Whatman, Maidstone, UK) using a tissue harvester (Brandel, Inc., Gaithersburg, MD). Radioactivity was quantified by liquid scintillation counting. Nonspecific binding of $[^3H]CPX$ was defined as $[^3H]CPX$ bound in the presence of 10 μM $N^0$-cyclopentyltheophylline. Triplet determinations were performed for each concentration of unlabeled compounds.

Effects of CVT-510 on Heart Rate and Serum NEFA Concentration in Awake Rats. Heart rate was measured from rats chronically instrumented with telemetry transmitters. For transmitter implantation, a midline laparotomy was performed on anesthetized rats and a transmitter for ECG recording was sutured to the abdominal wall. The two electrocardiographic leads were tunnelled...
through the wall, passed subcutaneously (one to the left shoulder, the other to the right thigh), and secured in place with sutures. Heart rates of awake rats were measured using a Dataquest A/R Gold System (Data Sciences International, St. Paul, MN). Cardiac electrical activity was recorded for 10-s periods and used to calculate heart rate in beats per minute. After recording of a baseline heart rate, either vehicle (0.9% DMSO in saline, 0.5 ml) or CVT-510 was injected into the intraperitoneal cavity of each rat, and heart rate was monitored at intervals for an additional 3 h.

The effects of CVT-510 on heart rate and to reduce serum NEFA concentration were determined in separate groups of rats to avoid the effects of animal handling and blood sampling on heart rate. Three days before an experiment, a catheter (0.025-mm outer diameter) was implanted in the left common carotid artery of each rat using aseptic conditions and sterile technique. The catheter was tunneled subcutaneously to the dorsal surface. After recovery from anesthesia, rats were placed in metabolic cages to facilitate handling and blood sampling. Blood samples (0.2 ml) were drawn before and at various time points after i.p. injection of either CVT-510 or vehicle (DMSO in saline). A 0.4-ml volume of 1% sodium citrate in saline was administered after withdrawal of each blood sample to replace blood volume and prevent clotting in the carotid artery catheter. Serum was collected from each sample after centrifugation of the clotted blood. Serum samples were stored at −80°C until analysis. Serum NEFA concentration was determined using an enzymatic colorimetric assay kit (Wako Chemicals, Richmond, VA).

The antilipolytic properties of CVT-510 in rats with catecholamines-stimulated NEFA levels were studied in rats with indwelling catheters (described above). Norepinephrine was delivered either by i.v. infusion into the jugular vein at 3 μg/kg/min for 30 min or by i.p. injection (60 μg/kg). CVT-510 was delivered by i.p. injection either before or after norepinephrine to determine whether the increase in NEFA concentrations caused by norepinephrine could be prevented or reversed by CVT-510.

Effects of Lowering NEFA Concentrations on Myocardial Oxygen Consumption. To assess the functional consequences of the antilipolytic effect of CVT-510, hearts isolated from rats were perfused in Langendorff mode at a constant flow of 10 ml/minute. Hearts were perfused with a modified Krebs’ solution (containing 118.0 mM NaCl, 4.7 mM KCl, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 2.5 mM CaCl₂, 25.0 mM NaHCO₃) supplemented with 0.2 or 0.8 mM palmitate to prevent clotting in the carotid artery catheter. Serum containing 0.8 mM palmitate then switched to solution containing 0.2 mM palmitate. The solutions were oxygenated with a 95% O₂ and 5% CO₂ gas mixture and maintained at 35°C. MVO₂ was determined from the arteriovenous difference in oxygen tension in the perfusate and pulmonary artery effluent respectively and calculated as follows: MVO₂ = (PAO₂ - PVO₂) × CP × c/760 × 100/g dry wt (Schenkman, 2001), where CP is the coronary flow (10 ml/min) and c is the solubility of oxygen in a physiological solution resembling plasma at 35°C (0.022) (Altman and Dittmer, 1971). The arteriovenous difference in oxygen tension was measured using flow-through oxygen electrodes (Microelectrodes, Inc., Bedford, NH). The oxygen pressure of the perfusate was calibrated in the probes (in series) at a constant flow of 10 ml/min with an ABL77 blood gas analyzer (Radiometer America, Inc., Westlake, OH). Similar experiments were performed in the reverse order of palmitate (0.2–0.8 mM) to account for possible storage of triglycerides that might occur in the opposite direction. Data were averaged for the two series.

Statistical Analyses. Data are presented as mean ± S.E.M. Data from cAMP accumulation and radioligand binding assays were analyzed using the GraphPad Prism software (GraphPad Software Inc., San Diego, CA) to determine the values of EC₅₀ (cAMP assay), IC₅₀ (inhibition of isoproterenol-induced cAMP accumulation by CVT-510), and Kᵢ (radioligand binding assay). A change in MVO₂ was assessed using a paired t test. Differences were deemed statistical if P < 0.05.

Results

Affinity and Potency of CVT-510 for A₁ Adenosine Receptors in Rat Adipocytes. The affinity of CVT-510 for A₁ adenosine receptors in membranes prepared from rat isolated adipocytes was determined by competition radioligand binding assays. Like the prototypical A₁-selective agonist R-PIA, CVT-510 reduced the binding of [³H]CPX to rat adipocyte membranes with a Kᵢ value of 11 nM (Fig. 1A) compared with 1.5 nM for R-PIA. Assays were carried out in the presence of the nonhydrolysable GTP analog guanosine 5’-(β,γ-imido)triphosphate (100 μM) to uncouple receptors from G proteins. Hence, the Kᵢ value represents the affinity of CVT-510 for A₁ receptors in their low-affinity state.

The potency of CVT-510 to inhibit isoproterenol-stimulated cAMP accumulation in rat isolated adipocytes was determined and compared with that of the high-affinity A₁ agonist R-PIA. As shown in Fig. 1B, both R-PIA and CVT-510 reduced the cellular content of cAMP in a concentration-dependent manner. The pEC₅₀ values for R-PIA and CVT-510 were 9.07 ± 0.03 and 8.89 ± 0.22 nM, respectively.

![Fig. 1.](image-url)
In Vivo Determination of the Antilipolytic and Bradycardic Effects of CVT-510. To determine the antilipolytic effects of the A1-selective agonist CVT-510 in awake rats, serum NEFA concentrations were measured and decreased concentrations were used as estimates of lipolysis inhibition. Figure 2A depicts the time- and dose-dependent effects of CVT-510 on serum NEFA concentrations. Each injection (2, 5, 20, and 50 μg/kg i.p.) caused a significant reduction of serum NEFA concentration. The baseline NEFA concentrations were 0.50 ± 0.02 mM. The maximal effect, as well as the time to full recovery (return to baseline value; vehicle-treated group), was dose-dependent. CVT-510, at 50 μg/kg, caused a prolonged (up to 3 h) reduction of serum NEFA concentration. The maximal effect (80% reduction of serum NEFA concentrations) was reached at 60 min after injection and declined gradually thereafter.

To determine the effect of CVT-510 on heart rate, heart rate was measured from telemetry-instrumented rats in the absence and presence of various doses of CVT-510. No significant reduction of heart rate was observed with CVT-510 at doses of 5 and 20 μg/kg (Fig. 2B). However, CVT-510 given at 50 μg/kg caused a significant bradycardia (50% decrease in heart rate at 25 min; Fig. 2B). Baseline heart rate was 365 ± 5 beats/min. To facilitate comparison of doses of CVT-510 on serum NEFA concentrations and heart rate, data for individual doses in Fig. 2 were replotted on separate axes in Fig. 3. Whereas the doses of 5 or 20 μg/kg CVT-510 had no effect on heart rate, they caused a marked reduction of serum NEFA. CVT-510 at 50 μg/kg caused a significant reduction of both heart rate and serum NEFA. However, as shown in Fig. 3C, the antilipolytic effect of 50 μg/kg CVT-510 lasted much longer than its bradycardic effect (3 versus 1 h).

Inhibition of Norepinephrine-Induced Lipolysis by CVT-510. The effect of A1 adenosine receptor activation on the lipolytic effect of NE was determined. As shown in Fig. 4, NE administered either by i.v. infusion (3 μg/kg/min for 30 min; Fig. 4A) or by i.p. injection (60 μg/kg; Fig. 4B) caused a significant increase of serum NEFA concentration (0.42–0.70 mM by i.v. or 0.5–0.9 mM by i.p.). This effect was completely prevented or reversed by i.p. injection of CVT-510 (20 μg/kg or 10 μg/kg) before or after NE treatment, respectively (Fig. 4, A and B). There was no effect on heart rate or blood pressure by NE either alone or in combination with CVT-510 compared with saline vehicle (data not shown).

In Vivo Antilipolytic Effect of CVT-510 Is Mediated by A1 Adenosine Receptors. To confirm that the antilipolytic effect of CVT-510 is mediated by A1 adenosine receptors,
the effect of CVT-510 on NEFA in the presence of the highly selective and potent A₁ antagonist CVT-124 (Belardinelli et al., 1995) was determined. As shown in Fig. 5, i.v. injection of CVT-124 (1 mg/kg) 20 min after i.p. injection of CVT-510 (20 μg/kg) rapidly and completely reversed the antilipolytic effect of CVT-510. CVT-124 (1 mg/kg i.v.) alone had no effect on plasma NEFA concentrations.

Effect of Decreasing Fatty Acid Concentration on Myocardial Oxygen Consumption in Rat Isolated Heart. To determine whether the antilipolytic activity of CVT-510 could have functional consequences in the heart, isolated hearts were exposed to concentrations of fatty acid (palmitate) that mimicked the in vivo fatty acid response to CVT-510, namely, 0.8 and 0.2 mM, in the absence and presence of CVT-510, respectively. MVO₂ significantly decreased by 6.25% (p = 0.001; n = 5) when the palmitate concentration in the perfusate was reduced from 0.8 to 0.2 mM, and significantly increased by 19% (p = 0.04; n = 3) when the palmitate concentration was increased from 0.2 to 0.8 mM. As shown in Fig. 6, the combined data yielded a net effect of a 10.9% change in MVO₂ (p = 0.004; n = 8).

Discussion

The development of direct-acting adenosine receptor agonists as potential therapeutic molecules has been particularly challenging due to the ubiquitous distribution of adenosine receptors, and consequently high potential for effects in organs/cells other than the target tissue. Adenosine agonists will activate all adenosine receptors (A₁, A₂A, A₂B, and A₃) to which they gain access and hence, elicit a wide range of effects. Not withstanding these challenges, in the present study we demonstrated that it is possible to selectively target the antilipolytic effect of an A₁ receptor agonist, CVT-510, independent of its cardiovascular effects.

The main goals of this study were 1) to determine whether the antilipolytic effect of the A₁-selective adenosine receptor agonist CVT-510 (Snowdy et al., 1999) could be differentiated from its negative chronotropic effects, 2) to determine whether CVT-510 could reverse a norepinephrine-induced
stimulation of serum NEFA concentrations in awake rats, and 3) to identify a meaningful functional consequence in cardiac tissue that would provide evidence that the antilipolytic actions of CVT-510 could be beneficial to the heart.

In the present study, we demonstrated that CVT-510, despite being a full and potent A₁ adenosine receptor agonist, can cause near-maximal reduction of NEFA concentration at doses that have little or no significant effect on heart rate. Although it has been long known that A₁ agonists are potent and efficacious inhibitors of lipolysis, limited efforts have been directed at the development of A₁ agonists as potential therapeutic antilipolytic agents, mainly due to the potential for adverse cardiovascular effects. Previous work with GR79236, an A₁ agonist, revealed that with this agonist it was not possible to obtain a substantial separation between its lipid-lowering and cardiovascular effects (Merkel et al., 1995). With CVT-510, NEFA levels can be lowered by approximately 60 to 70% at doses that this agonist does not cause bradycardia, whereas with GR79236, at concentrations that reduced glycerol 60%, heart rate was lowered approximately 30% (Merkel et al., 1995). Work by van Schaick et al. (1997) on the other hand, with N-6-(p-sulphophenyl) adenosine did show some tissue selectivity in vivo (van Schaick et al., 1997). Our data with CVT-510 not only shows that it is indeed possible to separate the NEFA-lowering from the bradycardic effects but also it demonstrates a beneficial cardiac consequence of the antilipolytic effect, that is, a decrease in MVO₂.

The use of R-PIA in these studies was not intended as a direct comparison for CVT-510, but rather as a positive control as R-PIA has been used extensively to investigate the antilipolytic effects of adenosine.

The separation of the antilipolytic effect from the cardiovascular effects of CVT-510 at low doses apparently results from the much higher sensitivity of adipose tissue to the selective A₁, adenosine receptor agonist. One possible explanation for the differential sensitivity is the existence of an adipose-tissue specific A₁ receptor subtype that is different from the heart. However, the results of a recent study by Tatsis-Kotidis et al. (1999) suggests that this is not the case. The sequences as well as the pharmacological properties of A₁ receptors cloned from mouse and human adipocytes are identical to those cloned from other tissues within the same species (Tatsis-Kotsis and Erlanger, 1999). The most likely explanation for the differences in sensitivity of the heart and adipocytes to CVT-510 is the different coupling efficiency of the A₁ agonist between the two tissues. Using an irreversible antagonist, Liang et al. (2002) demonstrated that occupancy of a very small fraction (<1%) of A₁ receptors expressed on adipocytes is sufficient to elicit near maximal response. Hence, there is a larger receptor reserve for A₁ receptor-mediated inhibition of lipolysis than cardiac function, probably due to the presence of a higher A₁ receptor density and more efficient receptor-effector coupling in adipose tissue than in the heart (Srinivas et al., 1997). Therefore, it should be possible to achieve maximal or near maximal antilipolytic effect with minimal or no cardiovascular effects by exploiting this differential receptor-effector coupling between adipose and cardiac tissue.

Receptor desensitization is another pharmacological phenomenon that needs to be circumvented with the chronic use of agonists. Extensive studies have concluded that the A₁ receptor can undergo agonist-induced long-term desensitization. However, unlike many G protein-coupled receptors, A₁ receptors are not subject to rapid (acute or short-term) desensitization probably due to the lack of agonist-dependent receptor phosphorylation (Gao et al., 1999). The desensitization of A₁ adenosine receptors, which requires prolonged exposure to high concentrations of agonists, results in down-regulation of receptors, G proteins and impaired receptor-effector coupling. In animals, the desensitization of A₁ receptor-mediated antilipolytic response occurred only after continuous subcutaneous delivery of high doses of PIA for several days (Hoffman et al., 1986). The presence of a large receptor reserve in adipose tissue makes it possible to achieve tissue selectivity while maintaining a maximal antilipolytic effect with relatively low concentration of a full agonist, or better yet with a partial agonist. This may minimize or help avoid unwanted receptor desensitization. Zannikos et al. (2001) recently reported rapid acute tolerance to the plasma NEFA-lowering effect of the reported adenosine receptor agonist after intravenous administration to fasted healthy volunteers. However, this finding is confounded by the fact that adenosine receptor agonist is not a selective A₁ agonist. In fact, it is a high-affinity agonist for both A₁ and A₂a adenosine receptors (Zannikos et al., 2001). Because activation of A₂a receptors can lead to sympatho-excitation (Fresco et al., 2002), including release of NE, lipolysis would be increased, and hence, counteract the antilipolytic effect mediated by A₁ receptor activation. Whether the increase in sympathetic drive can fully explain the apparent rapid loss of A₁ receptor-mediated antilipolytic effect remains to be determined.

Results from a clinical study in which CVT-510 was administered by an intravenous infusion (5–7.5 μg/kg + 0.1–0.2 μg/kg/min) to seven volunteer patients revealed that this A₁ agonist is capable of causing a sustained lowering of serum NEFA concentration (from ~0.65 to ~0.2 mM) during the entire time course of a 48-h infusion without causing bradycardia and or changes in blood pressure. This suggests that A₁ receptor desensitization did not occur. The return of serum NEFA concentrations after the cessation of CVT-510 administration which paralleled the decline of plasma CVT-510 concentrations supports the interpretation that the antilipolytic effects in these patients was indeed due to the effect of CVT-510 (CV Therapeutics, Inc., unpublished data on file). These results are in keeping with the present study in awake rats.

An additional concern over the use of A₁ agonists as long-term antilipolytic agents is the potential weight gain as a result of sustained suppression of lipolysis. Although this issue was not addressed in the present study, the results of a recent study by Dong et al. (2001) shed some light on this important question. Transgenic mice that overexpressed A₁ receptors specifically in adipose tissue had lower plasma NEFA concentrations but no discernible increase in body weight (Dong et al., 2001).

Elevated free fatty acid concentrations in the blood increase fatty acid oxidation in the heart; however, the process of fatty acid oxidation is less efficient than the oxidation of other substrates such as glucose as more oxygen is utilized per mole of fatty acid oxidized than per mole of glucose oxidized for the generation of ATP (for review, see Opie, 1998). Therefore, decreasing the reliance of the heart on fatty acids is expected to improve cardiac function. This would be
especially true during myocardial ischemia or surgery where serum NEFA concentrations are elevated (Hirvonen et al., 1978; Lopaschuk et al., 1994). To assess the potential for a decrease in serum NEFA to change MVO₂, isolated hearts were perfused with concentrations of fatty acids that mimicked the effect of CVT-510 to lower serum NEFA concentrations, namely, 0.8 and 0.2 mM. Decreasing fatty acid concentration in the perfusate decreased MVO₂, whereas increasing fatty acid concentration in the perfusate increased MVO₂. The net effect on MVO₂ was greater with increasing, than decreasing, fatty acid concentration likely due to accumulation of fatty acids in the form of triglycerides after perfusion with the high concentration of fatty acids. Regardless, on average, an 11% change in MVO₂ was observed. Albeit indirect, this finding is highly supportive of a switch from fatty acid to glucose oxidation and is in agreement with published rect, this finding is highly supportive of a switch from fatty acid to glucose oxidation and is in agreement with published

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Li, and Zablocki JA, Palle V, and Shryock JC (2001) A partial agonist (CVT-510, at doses that do not cause adverse cardiac effects, would lead indirectly to improved myocardial efficiency. Another expected desirable effect of lowering serum NEFA is increased insulin sensitivity (Dong et al., 2001). In the present study, we did not measure this effect of CVT-510. Whether these observations would be true during prolonged treatment remains to be determined; however, because the antilipolytic effect of CVT-510 in humans was found to be sustained (CV Therapeutics, Inc., unpublished data on file), it is plausible that its cardiac and metabolic effects, by lowering MVO₂ (shown in this study) and increasing insulin sensitivity (Dong et al., 2001) will also be sustained.