Demonstration of potent lipid-lowering activity by a thyromimetic agent devoid of cardiovascular and thermogenic effects


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Abstract

A potent lipid-lowering thyromimetic (CGS 26214) devoid of cardiac and thermogenic activity was identified based on its ability to preferentially access and bind the nuclear fraction of hepatocytes over that of myocytes in culture. The difference in access achieved with CGS 26214 was at least 100-fold better for hepatocytes than for myocytes. This in vitro hepatoselectivity resulted in a compound with unprecedented in vivo lipid-lowering potency with a minimal effective dose of 1 µg/kg in rats and dogs (~25x that of L-T3). At the same time, CGS 26214 was free of any cardiovascular effects up to the highest dose tested of 25 mg/kg and 100 µg/kg in rats and dogs, respectively.

Keywords: Hypolipidemic; L-T3; Cholesterol; LDL; Thyromimetic; Atherosclerosis

1. Introduction

Elevated serum cholesterol, especially LDL, is a well established risk factor for coronary heart disease (CHD) [1]. Drugs that lower serum LDL have shown a clear benefit in reducing morbidity and mortality rate due to CHD [2]. Current agents which effectively lower serum LDL do so by upregulating LDL receptor activity secondary to reduction of intracellular cholesterol [3] either by inhibiting cholesterol biosynthesis (HMG CoA reductase inhibitors) or by enhancing the conversion of intracellular cholesterol to bile acids (bile acid sequestrants).

Thyroid hormone (L-T3) is the most potent agent known to reduce serum LDL. While its efficacy is primarily attributed to the stimulation...
of LDL receptor activity [4–7], the molecular mechanism of this effect involves direct enhancement of LDL receptor gene transcription [8]. However, the use of thyroid hormone or its analogs as lipid-lowering drugs has not been possible due to the severe cardiovascular (CV) stimulation associated with its mode of action [9–12].

The cause of CV stimulation by thyroid hormone has been a source of considerable debate. While some human and animal studies have demonstrated direct effects of thyroid hormone on the heart, others have indicated that the cardiac effects were secondary consequences of the well-documented enhanced basal metabolic rate produced by thyroid hormone which brings about an increased demand for oxygen and blood supply to these tissues [12–14]. Therefore, a thyromimetic clinically useful as a hypolipidemic agent should be devoid of both direct cardiac effects and thermogenic activity.

The studies presented herein demonstrate that the novel thyroid hormone analog, CGS 26214 (Fig. 1), exhibits the lipid lowering properties of thyroid hormone yet is free of the cardiovascular and thermogenic side effects characteristic of thyroid hormone. These data as well as data related to the mechanism of separation of these activities in vitro and in vivo are presented.

2. Materials and methods

2.1. Materials

CGS 26214, {[4-3-(4-fluoro-α-hydroxybenzyl)-4-hydroxyphenoxy]-3,5-dimethyl[phenyl]amino} -oxoacetate, was synthesized in our laboratories at Ciba Pharmaceuticals (Summit, NJ). [125I]Labeled L-T3 and [125I]labeled LDL were obtained from New England Nuclear (Boston, MA) and Biomedical Technologies (Stoughton, MA), respectively. Tissue culture reagents were obtained from Gibco (Grand Island, NY) and all other chemicals were purchased from Sigma (St. Louis, MO). Rat chow supplemented with 1.5% cholesterol and 0.5% cholic acid was supplied by Dyets (Bethlehem, PA).

2.2. Binding to nuclear L-T3 receptor

Rat liver and heart nuclei were obtained from male Sprague-Dawley rats (Tac:N(SD)fBr). The rat liver nuclear fraction was obtained in the 250 × g sediment following the procedure of Emmelot et al. [15]. The nuclei within this fraction were isolated and purified as described previously by Spindler et al. [16]. The rat cardiac nuclear receptors were isolated non-enzymatically as described by Jackowski and Liew [17]. The hearts were excised from adult male Sprague-Dawley rats and rinsed in ice-cold TMNC-PMSF buffer (10 mM Tris-HCl, 10 mM NaCl, 1 mM MgCl2, 0.1 mM phenylmethylsulphonylfluoride, pH 7.4). The aorta and atria were dissected free and the ventricles weighed (14.4 g), minced with scissors, and separated into 2 g portions. Each portion was homogenized for 5 s in 20 ml of MA buffer (0.25 M sucrose, 10 mM Tris-HCl, 3 mM MgCl2, 0.1 mM phenylmethylsulphonylfluoride, pH 7.4) using setting 4 of a Polytron homogenizer and then centrifuged at 800 × g for 10 min. The resultant pellet was resuspended in 10 vol (20 ml) of MA buffer and homogenized in a Dounce homogenizer with 6 gentle strokes of the loose pellet. It was then filtered through one layer of spectra/mesh fluorocarbon filter with a mesh opening of 70 μm. The sample was then recentrifuged at 800 × g for 10 min, and all of the pellets from each aliquot were washed with 90 ml of 0.5% Triton.
X-100 in MA buffer by centrifugation at 800 × g for 10 min. The later step was repeated and then the nuclear pellets were resuspended in 23 ml of MC buffer (2.2 M sucrose, 10 mM Tris-HCl, 1 mM MgCl₂·6H₂O, 0.1 mM phenylmethylsulphonylfluoride, pH 7.4) and layered over a discontinuous sucrose gradient consisting of 5 ml of 2.3 M and 8 ml of 2.7 M sucrose in MWS buffer (10 mM Tris-HCl, 1 mM MgCl₂·6H₂O, 0.1 mM phenylmethylsulphonylfluoride, pH 7.4). The gradient was subjected to centrifugation at 12 700 × g for 1 h and the nuclear band at the 2.3 M/2.7 M sucrose interface was recovered (myocyte nuclei). The resultant nuclei were washed in 180 ml of reaction buffer (20 mM Tris-HCl, 0.25 M sucrose, 1 mM MgCl₂·6H₂O, 2.0 mM EDTA, 0.1 mM DTT, 50 mM NaCl, 5% glycerol, pH 7.2) by centrifugation at 12 700 × g for 25 min. The nuclear pellets obtained were resuspended in 1.7 ml of reaction buffer and snap-frozen in a dry ice/acetone bath and stored frozen at −40°C.

Nuclear binding was determined according to the method of Spindler et al. [16] with minor modifications. To measure total binding, nuclei (300 μg of protein) were incubated with 0.3 nM [³²P]L-T₃ (1080 mCi/mg) for 50 min at 22°C in a final volume of 1.0 ml of buffer A consisting of 20 mM Tris-HCl, 0.25 M sucrose, 1 mM MgCl₂·6H₂O, 2.0 mM EDTA, 0.1 mM dithiothreitol, 50 mM NaCl and 5% glycerol (pH 7.2). Parallel incubations were conducted with tubes containing in addition to the nuclear suspensions and radioactive L-T₃, either various concentrations of the test compounds or excess of unlabeled L-T₃ (3 μM). The latter served as a measure of non-specific binding. Following incubation, the samples were chilled in an ice bath and centrifuged at 800 × g for 7 min at 4°C. The pellet was washed by suspending it in 2 ml of buffer B (buffer A with 0.5% Triton X-100; pH 7.2) and mixing for 5 s. Tubes were then centrifuged at 800 × g for 7 min at 4°C. The supernatant was aspirated off and the pellet was washed again and re-centrifuged as described above. Radioactivity in the pellet was determined in a LKB 1282 γ-counter. Specific binding was calculated as the difference between total binding (incubation with excess unlabeled L-T₃) and non-specific binding (incubation with excess unlabeled L-T₃). The concentration of test compounds corresponding to half-maximal inhibition (IC₅₀) of specific binding of [³²P]L-T₃ was determined from the reciprocal plot of specific binding vs. concentration of the test compound.

2.3. Cholesterol-lowering studies

Euthyroid male Sprague-Dawley rats (230–250 g) were maintained ad libitum on water and rat chow diet containing 1.5% cholesterol and 0.5% cholic acid for 2 weeks prior to and during the 7-day treatment period. Groups of six animals were treated orally once a day with vehicle (water) alone or with the test compounds for 7 consecutive days. After the last dose, animals were fasted for 18 h and blood was collected by cardiac puncture under CO₂ anesthesia. Blood was withdrawn in 5% EDTA (50 μl/ml blood) and plasma was prepared by centrifugation at 2500 rpm for 10 min at 4°C. Samples were analyzed enzymatically for plasma total and HDL cholesterol on a BioMek automated work station (Beckman Instruments, Palo Alto, CA) using a Sigma diagnostic reagent kit.

Euthyroid male beagle dogs (Marshall Farms, North Rose, NY) with LDL cholesterol concentrations ranging from 12 to 17 mg/dl were selected. Groups of six dogs were subjected to a dosing regimen in which each dog served as its own control. The dogs were treated with test compounds for 5 consecutive days administered by gastric gavage. The dogs were bled from the jugular vein prior to the first dose and 2 h following the last dose. Plasma total, HDL cholesterol and triglyceride concentrations were determined as described above.

2.4. LDL receptor studies

Binding of [³²P]LDL to rat liver membrane LDL receptors was analyzed as described by the method of Kovanen et al. [18] using liver membrane from male rats treated with either vehicle or test compounds orally for 7 days. LDL receptor activity in HepG2 cells was determined in cells grown in Dulbecco's modified Eagle's medium.
(DMEM) supplemented with 10% fetal calf serum (FCS). After 72 h, the medium was changed to DMEM without FCS for 24 h prior to changing to DMEM containing either vehicle or test compound for an additional 48 h. Cell-associated LDL was determined after incubation at 37°C for 2 h with DiI-LDL. At the end of the incubation period, cells were extensively washed with PBS and then extracted with isopropanol for measurement of DiI fluorescence as previously described [19], and cell protein was assayed after NaOH digestion according to Bradford [20].

2.5. Transport studies in intact cells

Binding affinity of L-T₃ and CGS 26214 was studied in nuclei of intact HepG2 cells and neonatal rat cardiac myocytes. HepG2 cells were purchased from American Tissue Type Culture Collection (Bethesda, MD). Rat neonatal myocytes were isolated from 3-day old Sprague-Dawley rat pups and plated in 60 mm Falcon Primaria culture dishes according to the method of Janero et al. [21]. Myocytes were differentiated from fibroblasts using a standard differential attachment procedure, and the purified myocytes were replated. Myocytes were incubated for 3 days and used on the 3rd and 4th day for experiments. For transport studies, the growth medium (Dulbecco’s Modified Eagle Media, DMEM, with 10% fetal calf serum) was replaced with serum-free medium containing [¹²⁵I]L-T₃ (0.08–0.24 nM) plus various concentrations of unlabeled L-T₃ or CGS 26214 (10⁻⁶–10⁻¹² M). The cells were incubated for 1 h at 37°C, thoroughly washed (3x) with buffer (Hanks’ Balanced Salt solution minus Mg²⁺ and Ca²⁺) and resuspended in the same buffer. The radioactivity for each cell suspension was measured to determine total cell equilibrium uptake. Subsequently, the cell suspension was centrifuged at 300 × g, the supernatant was discarded and the cell pellet was resuspended in a hypotonic buffer (Sucrose, 250 mM; Tris-HCl, 20 mM; DTT, 1.0 mM; PMSF, 0.10 mM; CaCl₂, 1.0 mM; MgCl₂, 1.0 mM; pH 7.8) containing 1% Triton X-100 and incubated in an ice bath for 15 min. The nuclear pellet was collected by centrifugation, and after several washes, the radioactivity of each nuclear pellet was measured to determine nuclear binding. Direct measurement of cellular uptake of L-T₃ and CGS 26214 was determined by the procedure described above using 0.1 μM [¹⁴C]CGS 26214 or [¹²⁵I]L-T₃ without addition of unlabeled ligand.

2.6. Cardiovascular studies

Euthyroid male Sprague-Dawley rats (235–265 g) were maintained ad libitum on standard chow and water. Groups of six animals were treated orally as described above. On day 8, animals were killed by decapitation. Hearts were quickly removed and rinsed in 20 ml of tissue bath containing Krebs buffer gassed with 95% O₂, 5% CO₂ at 28°C. Right atria were dissected, hung with minimum applied tension and allowed to equilibrate for 30 min. The spontaneous atrial rate was then determined. Left atria were dissected from the heart and mounted on platinum electrodes positioned in tissue baths containing Krebs buffer. The resting tension was set at 2 g. Left atria were stimulated at 10 beats/min (bpm) with a minimum stimulus duration of 2.5 ms. Atria were then allowed to equilibrate for 30 min. Tension-force curves were generated by altering tension and measuring the change in developed contractile force as described by Murayama and Goodkind [22]. The maximum developed contractile force was measured for each atrium. Isometric contractile forces were measured at frequencies of 10, 20, 40 and 80 bpm. Rat total body and heart weights were recorded to calculate relative heart weight.

Mixed-breed dogs were instrumented using sterile techniques under general anesthesia (pentobarbital sodium 25 mg/kg). An electromagnetic flow transducer (Carolina Medical Electronics) was placed around the ascending aorta for the measurement of cardiac output. To measure left ventricular pressure, catheters were placed in the aorta, the left atrium and in the right ventricle. A Konigsberg pressure gauge was placed in the apex of the left ventricle for the measurement of left ventricular (LV) pressure and LV dP/dt. Experiments were started when the dogs were fully trained to lie unrestrained on the laboratory table and hemodynamic parameters were recorded with...
Table 1
<table>
<thead>
<tr>
<th>Compound</th>
<th>(IC\textsubscript{50} nM)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
</tr>
<tr>
<td>L-T\textsubscript{3}</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>CGS 26214</td>
<td>0.1 ± 0.02*</td>
</tr>
</tbody>
</table>

The concentration of the compound corresponding to half-maximal inhibition (IC\textsubscript{50}) of specific binding of \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) was determined from the reciprocal plot of specific binding vs. concentration of test compounds. Intact nuclei were isolated from adult male rat liver and heart ventricles using non-enzymatic procedures as described in Section 2.

Values are mean ± S.E.M.; n = 6 determinations per group.

*Statistically different from L-T\textsubscript{3} treatment groups by Student’s t-test (P < 0.05).

In competitive binding studies using intact HepG2 cells and neonatal rat cardiac myocytes, there was no significant difference between the ability of L-T\textsubscript{3} to displace its labeled ligand from either nuclear fractions (Table 2); however, CGS 26214 was over 200-fold more effective in competing with \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) for nuclear receptor binding in the intact HepG2 cells compared to neonatal rat cardiac myocytes (Table 2). A similar relationship was observed when CGS 26214 was compared to D-T\textsubscript{3} which competed less effectively than its l-enantiomer in both cell types. Moreover, D-T\textsubscript{3} was less effective in competing for the nuclear receptor of liver cells but more effective in competing for that of cardiac myocytes than CGS 26214 (Fig. 2).

When \([^{14}C]\text{CGS} 26214\) (0.1 mM) and \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) (0.1 mM) were incubated with neonatal cardiac myocytes and HepG2 cells in the absence of non-radioactive ligand, the uptake of \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) by cardiac myocytes was significantly more than that by HepG2 cells.

### 3. Results

#### 3.1. Thyroid hormone receptor binding and cellular vs. nuclear uptake studies

CGS 26214 effectively competed with \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) for binding to L-T\textsubscript{3} nuclear receptor isolated from rat liver. Under the same conditions in which 0.6 nM of L-T\textsubscript{3} inhibited \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) nuclear binding by 50% (IC\textsubscript{50}), CGS 26214 had an IC\textsubscript{50} of 0.1 nM (Table 1). When identical studies were performed using the nuclear fraction isolated from rat ventricular myocytes, similar results were obtained. L-T\textsubscript{3} exhibited an IC\textsubscript{50} of 0.4 nM and CGS 26214 had an IC\textsubscript{50} of 0.2 nM (Table 1). Thus, CGS 26214 was significantly (P < 0.05) more effective than the natural hormone in displacing \([^{125}]\text{I}-\text{L-T}\textsubscript{3}\) from its nuclear receptor isolated either from rat liver or cardiac ventricular myocytes.
Fig. 2. Competition of L-T₃ (□), D-T₃ (○) and CGS 26214 (△) with [³²P]L-T₃ for occupation of thyroid hormone nuclear receptors in vitro following incubations with intact (A) HepG2 cells or (B) newborn rat cardiac myocytes.

of [¹⁴C]CGS 26214 (P < 0.05) (Table 3). When the same experiments were repeated with HepG2 cells, the disparity between the two ligands was even greater than for myocytes, with the uptake of [¹²⁵I]L-T₃ being as much as eight times greater

Table 3
Cellular uptake of [¹²⁵I]L-T₃ and [¹⁴C]CGS 26214 by HepG2 cells and neonatal rat cardiac myocytes

<table>
<thead>
<tr>
<th>Compound</th>
<th>HepG2 (fmole/mg cell protein)</th>
<th>Myocyte (fmole/mg cell protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-T₃</td>
<td>62 ± 2</td>
<td>64 ± 2</td>
</tr>
<tr>
<td>CGS 26214</td>
<td>7.2 ± 0.9*</td>
<td>14.4 ± 0.5*</td>
</tr>
</tbody>
</table>

Cellular uptake was measured in cells after incubation with radiolabeled CGS 26214 and L-T₃ without addition of unlabeled ligand.

Values are mean ± S.E.M.; n = 6 per group.

*Statistically different from myocytes by Student’s t-test (P < 0.05).

+Statistically different from L-T₃ treatment group by Student’s t-test (P < 0.05).

Table 4
Cholesterol-lowering activity of L-T₃ and CGS 26214 in rats

<table>
<thead>
<tr>
<th>Dose (µg/kg)</th>
<th>% Change from control</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>LDL</td>
</tr>
<tr>
<td>1</td>
<td>−3</td>
</tr>
<tr>
<td>3</td>
<td>−8</td>
</tr>
<tr>
<td>10</td>
<td>−21</td>
</tr>
<tr>
<td>30</td>
<td>−37*</td>
</tr>
<tr>
<td>100</td>
<td>−55*</td>
</tr>
</tbody>
</table>

Hypercholesterolemic rats were treated orally with test compounds for 7 days.

Values represent mean percent change from control; n = 6 per treatment group.

Control value for rat LDL = 153 mg/dl; HDL = 25 mg/dl.

*Statistically different from control by Student’s t-test (P < 0.05).

3.2. Cholesterol-lowering studies

In hyperlipidemic rats, LDL cholesterol was dose-dependently reduced by both CGS 26214 and L-T₃ (Table 4) with minimal effective doses of 1 µg/kg and 30 µg/kg, respectively, producing approximately 35% reduction. HDL cholesterol was not affected by either compound up to the highest dose tested of 100 µg/kg. In normolipidemic dogs, CGS 26214 was tested in animals used as their own control (Table 5). Similar to the effects seen in rats, LDL was dose-dependently reduced by CGS 26214 with a minimum effective dose (MED) of 1 µg/kg; HDL and triglycerides were not changed.

3.3. LDL receptor studies

The effect of CGS 26214 on LDL receptor activity was examined in HepG2 cells at 10 nM and in liver membrane prepared from rats treated with 10 µg/kg for 7 days. The results shown in Table 6 indicate that LDL receptor activity was stimulated by CGS 26214 in cells and liver membrane by 31% and 33%, respectively.
Table 5
Cholesterol-lowering activity of CGS 26214 in dogs

<table>
<thead>
<tr>
<th>Dose (µg/kg)</th>
<th>LDL (%)</th>
<th>HDL (%)</th>
<th>TG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-59*</td>
<td>-5</td>
<td>-5</td>
</tr>
<tr>
<td>3</td>
<td>-68*</td>
<td>0</td>
<td>+6</td>
</tr>
<tr>
<td>10</td>
<td>-86*</td>
<td>-4</td>
<td>+3</td>
</tr>
</tbody>
</table>

Normolipemic dogs were treated orally with the test compound for 7 days. Values represent mean percent change from baseline; n = 6 per treatment group. Baseline value for dog LDL = 15 mg/dl; HDL = 120 mg/dl; TG = 26 mg/dl. Statistically different from baseline by paired t-test (P < 0.05).

3.4. Cardiovascular studies

The cardiovascular (CV) effects of CGS 26214 were compared to those of L-T₃ in two different species, rats and dogs. In rats, atrial rate, atrial tension and heart weight were determined after oral administration of either CGS 26214 or L-T₃ for 7 days. The results shown in Fig. 3 indicate that all CV parameters were dose-dependently stimulated by L-T₃ with a MED estimated at 30 µg/kg. This dose was equal to the MED on serum cholesterol thus giving a therapeutic safety index of 1.00, i.e. no dissociation between hypolipidemic and CV effects. In sharp contrast to L-T₃, CGS 26214 was devoid of any CV effects up to the highest dose tested of 25 mg/kg which is > 25 000 times the MED on serum cholesterol thus giving an extremely wide margin of safety for this compound.

The CV safety of CGS 26214 was also evaluated in conscious, chronically instrumented dogs treated with 100 µg/kg for 7 days. This dose of CGS 26214 is equivalent to 100-fold its therapeutic lipid-lowering dose. As a positive control, L-T₃ given at only 10-fold its lipid-lowering dose, i.e. 300 µg/kg, produced all of its expected CV-related effects such as increased heart rate, cardiac output and dP/dt which is an indirect measure of
cardiac contractility, and in addition whole body oxygen consumption (Table 7). L-T3 also raised body temperature by about 1.14°C over the baseline value of 39°C. In contrast to L-T3, CGS 26214 administered at 100-fold the MED for serum cholesterol, i.e. 100 µg/kg, showed no significant effect on any of the CV parameters described above nor on body temperature.

4. Discussion

To determine the potential mechanism(s) responsible for the dissociation of lipid-lowering from CV effects, competitive binding studies were conducted comparing the binding of L-T3 and CGS 26214 to the thyroid hormone nuclear receptor in liver and heart tissues under various incubation conditions. Using a classical competitive binding assay which involved incubating the competing ligands with isolated nuclei, there was no significant difference in the binding of CGS 26214 to isolated nuclei from rat liver or heart tissues. However, when competitive binding studies were performed with intact human HepG2 cells and neonatal rat cardiac myocytes and their respective nuclear fractions were isolated and the [125I]L-T3 occupancy determined, the binding of CGS 26214 to nuclei from neonatal rat myocytes was markedly less than its previously noted binding to isolated rat myocyte nuclei, while its binding to the HepG2 cell nuclei did not differ from that previously observed using isolated nuclei from rat liver. These data indicate that CGS 26214 was not able to access the nuclei of myocytes as well as those of the hepatocytes. This difference was unique to CGS 26214, in that L-T3 bound equally well to the isolated nuclei from hepatocytes and myocytes compared to the whole cell nuclei from HepG2 cells and neonatal rat myocytes. These findings are in agreement with those of Underwood et al. [24] who have previously shown that the cardiac-sparing, lipid-lowering thyromimetic SKF L-94901 bound equally well to isolated rat heart and liver nuclei, but when administered in vivo bound more efficiently to hepatic than to cardiac nuclei. Selective uptake of thyromimetics into the nuclei obtained from liver vs. heart tissues was originally demonstrated for the stereoisomers of L-T3. D-T3 administered in vivo exhibited a 5- to 6-fold preferential occupancy of liver vs. heart nuclei while L-T3 showed no tissue selectivity [25,26].

A possible explanation for the reduced nuclear binding between neonatal ventricular myocytes compared to intact HepG2 cells or isolated myocyte nuclei would be a reduced uptake of CGS 26214 into the myocyte vs. the HepG2 cell. However, our data indicate that myocytes actually incorporate more [14C]CGS 26214 than do HepG2 cells. Thus, the reduced nuclear receptor binding of CGS 26214 in cardiac myocytes could not be attributed to a reduced cellular uptake of CGS 26214. It should be noted that while the normal physiological levels of CGS 26214 are between 1–10 nM, the cellular uptake studies were conducted with 0.1 mM of either [14C]CGS 26214 or [125I]L-T3 because of the low specific activity of the [14C]CGS 26214. Consequently, because of this low specific activity it was not possible to detect nuclear uptake of [14C]CGS 26214 despite using these supraphysiological concentrations. It

Table 7
Effect of L-T3 and CGS 26214 on cardiovascular and hemodynamic parameters in conscious dogs

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-T3 (% Change from baseline)</th>
<th>CGS 26214</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (bpm)</td>
<td>+53* (94)</td>
<td>+15 (88)</td>
</tr>
<tr>
<td>CO (l/min)</td>
<td>+63* (4.0)</td>
<td>+19 (3.0)</td>
</tr>
<tr>
<td>WBOC (ml/min)</td>
<td>+47* (135)</td>
<td>+8 (146)</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>+6 (95)</td>
<td>-5 (110)</td>
</tr>
<tr>
<td>dP/dt (mmHg/s)</td>
<td>+27* (2.359)</td>
<td>NA</td>
</tr>
</tbody>
</table>

Dogs instrumented with electromagnetic flow transducer and pressure gauges at critical sites were treated orally with either L-T3 (300 µg/kg) or CGS 26214 (100 µg/kg) for 7 days. Cardiovascular and hemodynamic parameters were measured before and after treatment.

HR, heart rate; CO, cardiac output; WBOC, whole body oxygen consumption; MAP, mean arterial pressure; NA, data not available.

Values represent mean percent change in groups of six dogs. Values in parentheses represent baseline value for each parameter.

*Statistically different from baseline by paired t-test (P < 0.05)
is noteworthy that the low uptake of [14C]CGS 26214 by both cell types was not a result of saturation since neither [14C]CGS 26214 nor [125I]L-T₃ exhibited any concentration-dependent decrease in cellular uptake even at these high concentrations (data not shown).

Based on the data reported here, it is not possible to ascribe a mechanism for the low level of nuclear binding of CGS 26214 in intact cardiac myocytes. Because a reduced uptake in cardiac vs. liver cells was not observed, active transport at the cell membrane can not explain the differential binding noted with CGS 26214 between the two cell types. Likewise a difference in binding affinities for different isoforms of the nuclear receptor is extremely unlikely to explain the discrepancy between the two cell types since comparable binding affinity was obtained in the isolated nuclear binding studies for both cell types; both liver and heart cells contain the α₁ and β₁ isoforms [27], and to date no functional differences have been attributed to binding to either one of these isoforms [28].

Like thyroid hormone, CGS 26214 appears to selectively lower LDL cholesterol at least in part by enhancing LDL receptor activity. Consistent with previously reported studies using human cells exposed to thyroid hormone in vitro [4,29] and fibroblasts from humans of varying thyroid hormone exposure [30], CGS 26214 increased LDL receptor activity in cultured human HepG2 cells and in liver membranes isolated from rats treated with CGS 26214 in vivo. Furthermore, CGS 26214 reduced LDL cholesterol without affecting HDL in hypercholesterolemic rats fed a cholesterol-cholic acid diet that is known to selectively raise their LDL cholesterol levels. This LDL-selective lowering effect of CGS 26214 was also observed in normocholesterolemic dogs. The minimal effective doses for reducing LDL cholesterol in dogs and rats were at least two orders of magnitude less than the doses shown to be totally free of any cardiovascular effects. In contrast, L-T₃ demonstrated all of its expected thyromimetic cardiovascular effects in both species: i.e. increased heart rate, cardiac output and cardiac contractility and decreased systemic vascular resistance.

In addition to the cardiac-sparing effect of CGS 26214, the thermogenic effect which is a hallmark of thyromimetic activity in mammals was totally absent when measured in dogs. Whole body oxygen consumption was not affected in dogs treated with CGS 26214 while it was increased in the L-T₃-treated animals. Consequently, dogs treated with L-T₃ experienced a significant increase in body temperature over baseline which was not observed with CGS 26214. Since the thermogenic response of thyroid hormone can be ascribed to its action on specific tissues including the liver [31] which accounts for about 20% of whole body oxygen consumption [32], and since the thermogenic effect is believed to be mediated by the L-T₃ receptor [33], it is surprising that no increase in basal metabolic rate was noted in vivo with CGS 26214 which enhances other receptor mediated effects in the liver such as LDL receptor activity. However, recent reports [34,35] indicate that di-iodothyronines (3,3'- and 3,5-di-iodothyronines) are capable of a direct acute effect on hepatic mitochondrial respiration which differs from that of L-T₃ in timing and mechanism of action. Because thyroid hormone is metabolized to these di-iodothyronines, at least some of its metabolic activity may be attributable to their mitochondrial activities. It is unlikely for CGS 26214 to exhibit the mitochondrial activity displayed by the endogenous di-iodothyronines since its structure (Fig. 1) is not compatible with the structural requirements for the direct mitochondrial respiratory activity previously described [34], i.e. the bulky 3' arylmethyl substituent in CGS 26214. Additional studies will be needed to further define the metabolic anomalies of CGS 26214.

To conclude, the results presented in this report demonstrate that the selection of a thyromimetic with limited access into the nuclear fraction of non-hepatic cells such as myocytes, would be highly predictive of a cardiac-sparing lipid-lowering compound. The remarkable lipid-lowering potency obtained with CGS 26214 coupled with the high degree of dissociation from the non-desirable CV side-effects has resulted in a hypolipidemic agent with unprecedented preclinical efficacy and safety profile.
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