A quest for connections : ligands for the HCA2, adenosine A3 and GPR88 receptors

Blad, C.C.

Chapter 3

Effects of pyrazole partial agonists on HCA_2-mediated flushing and VLDL-triglyceride levels in mice

This chapter is based on: Li Z, Blad CC, van der Sluis RJ, de Vries H, Van Berkel TJC, IJzerman AP, Hoekstra M, Br J Pharmacol **2012**; 167(4): 818-25

Abstract

Background and purpose: Niacin can effectively treat dyslipidemic disorders. However, its clinical use is limited due to the cutaneous flushing mediated by the nicotinic acid receptor HCA_2. In the current study, we evaluated two partial agonists for HCA_2, LUF6281 and LUF6283, with respect to their anti-dyslipidemic potential and cutaneous flushing effect.

Experimental approach: In vitro potency and efficacy studies with niacin and the two HCA_2 partial agonists were performed using HEK293T cells stably expressing human HCA_2. Normolipidemic C57BL/6 mice received either niacin or HCA_2 partial agonists (400 mg/kg/day) once a day for 4 weeks for evaluation of their effects in vivo.

Key results: Radioligand competitive binding assay showed K_i values for LUF6281 and LUF6283 of 3 μM and 0.55 μM. [^{35}S]-GTPγS binding determined niacin > LUF6283 > LUF6281 as rank order of their potency. All three compounds similarly reduced plasma VLDL-triglyceride concentrations, while LUF6281 and LUF6283 - in contrast to niacin - did not also exhibit the unwanted flushing side effect in C57BL/6 mice. Niacin lowered the adipose tissue expression of lipolytic genes HSL and ATGL by 50% ($P<0.05$). LUF6281 and LUF6283 unexpectedly did not. In contrast, the decrease in the VLDL-triglyceride concentration upon LUF6281 and 6283 treatment was associated with a parallel >40% decrease ($P<0.05$) in the expression level of APOB within the liver.

Conclusions and Implications: The current study identifies LUF6281 and LUF6283, two HCA_2 partial agonists of the pyrazole class, as promising drug candidates to achieve optimal lipid-lowering while successfully avoiding the unwanted flushing side effect.

Introduction

Niacin, also known as nicotinic acid, is the most effective agent currently available to treat dyslipidemic disorders [1]. It lowers plasma levels of pro-atherogenic lipids, including chylomicrons, very-low-density lipoproteins (VLDL), low-density lipoproteins (LDL), and triglycerides (TG) in normolipidemic as well as hypercholesterolemic subjects [2]. Several clinical trials have shown that nicotinic acid reduces cardiovascular disease and myocardial infarction incidence, providing a solid rationale for the use of niacin in the treatment of atherosclerosis [3-4]. The G protein-coupled receptor GPR109A, also known as PUMA-G in mouse and HM74A in humans, has been identified as a high-affinity receptor for niacin [5-6]. We now know that the endogenous ligand for GPR109A is 3-hydroxybutyrate, and this receptor has recently been renamed as hydroxy-carboxylic acid receptor 2 (HCA_2) [7]. Despite its established cardiovascular benefits, the clinical use of niacin has been limited due to the cutaneous flushing, a well-recognized adverse skin effect from niacin therapy. Flushing has been cited as the major reason for the discontinuation of this therapy [8]. The nicotinic acid receptor HCA_2 expressed in the skin is a critical mediator of niacin-induced flushing [9]. Niacin stimulates HCA_2 in epidermal Langerhans cells and keratinocytes, causing the cells to produce vasodilatory prostaglandin D2 (PGD_2) and prostaglandin E2 (PGE_2), which leads to cutaneous vasodilation [10-13].

For the past decade, the pharmacology of HCA_2 has been studied and full or partial agonists for HCA_2 have been developed in an attempt to achieve the beneficial effects of niacin while avoiding the unwanted flushing side effect [14]. Based on the structure-activity relationship of niacin-related molecules, several potent agonists for HCA_2 have been identified, including acipimox, acifran, 3-pyridine-acetic acid, 5-methylnicotinic acid, pyridazine-4-carboxylic acid, and pyrazine-2-carboxylic acid [15-16]. However, the challenge remains that HCA_2 partial agonists failed to mimic the beneficial effects of niacin on LDL-cholesterol, triglycerides or HDL-cholesterol despite the absence of flushing events in clinical studies [17]. Further understanding of the medicinal chemistry of HCA_2 is needed to pharmacologically dissociate the anti-lipolytic and vasodilatory effects of niacin by acting on HCA_2.

In the current study, we assessed the properties of two HCA_2 partial agonists, LUF6281 and LUF6283, of the pyrazole class, which were developed in our laboratory [18]. We first characterized these two compounds in vitro, using a radioligand binding assay, [^{35}S]-GTPγS assay and ERK phosphorylation assay. The ERK phosphorylation assay was included because it has been suggested that ERK1/2 phosphorylation downstream from HCA_2 correlates positively with skin flushing [19]. Subsequently, we determined the cutaneous flushing effect and the lipid-lowering potential of these two partial agonists in normolipidemic C57BL/6 mice which represent a good mouse model to study the VLDL-triglyceride lowering effect of niacin [20].

Methods

Materials

[^{3}H]-nicotinic acid (60 Ci/mmol) was obtained from BioTrend (Köln, Germany). [^{35}S]-

GTPγS (1250 Ci/mmol) was obtained from Perkin Elmer (Waltham, MA).

Cell culture and membrane preparation

Human embryonic kidney (HEK) 293T cells stably expressing human HCA_2 were cultured in DMEM supplemented with 10% newborn bovine serum, 0.4 mg/mL G418, 50 IU/mL penicillin and 50 μg/mL streptomycin. The cells were harvested by scraping in cold PBS, centrifuged at 1000 x g for 10 minutes and resuspended in cold 50 mM Tris-HCl buffer, pH 7.4. Then a DIAX 900 electrical homogenizer (Heidolph, Schwabach, Germany) was used for 15 seconds to obtain cell lysis. The suspension was centrifuged at 225000 x g for 20 minutes at 4 °C and the supernatant was discarded. The pellet was resuspended in Tris-HCl, and the homogenization and centrifugation steps were repeated. The membranes were resuspended in cold assay buffer (50 mM Tris HCl, 1 mM $MgCl_2$, pH 7.4) and the protein content was determined using BCA assay (Thermo Scientific, Waltham, USA). During membrane preparation the suspension was kept on ice at all times. Membrane aliquots were stored at -80°C until the day of use.

[^{3}H]-nicotinic acid displacement assay

Membranes of our stable HEK293T-HCA_2 cell line (50 μg protein per tube) were incubated for 1 hour at 25°C with 20 nM [^{3}H]-nicotinic acid and with increasing concentrations of the test compounds in assay buffer (50 mM Tris HCl, 1 mM $MgCl_2$, pH 7.4). The total assay volume was 100 μL. To assess the total binding, a control without test compound was included. The non-specific binding was determined in the presence of 10 μM unlabeled nicotinic acid. Final DMSO concentration in all samples was ≤0.25%. The incubation was terminated by filtering over GF/B filters using a 24-sample harvester (Brandel, Gaithersburg, USA). The filters were washed 3 times with 2 mL cold buffer (50 mM Tris HCl, pH 7.4). Filters were transferred to counting vials and counted in a Perkin Elmer LSA Tri-Carb 2900TR counter after 2 hours of extraction in 3.5 mL Emulsifier Safe liquid scintillation cocktail (Perkin Elmer, Waltham, USA).

[^{35}S]-GTPS binding assay

This assay was performed in 96-well format in 50 mM Tris-HCl, 1 mM EDTA, 5 mM $MgCl_2$, 150 mM NaCl, pH 7.4 at 25°C with 1 mM DTT, 0.5% BSA and 50 μg/mL saponin freshly added. HEK-HCA_2 membranes (5 μg protein per well in 25 μL) were pre-incubated with 25 μL of 40 μM GDP and 25 μL of increasing concentrations of the test compounds, for 30 minutes at room temperature. Then, 25 μL [^{35}S]-GTPγS was added (final concentration 0.3 nM) and the mixture was incubated for 90 minutes at 25°C with constant shaking. The incubation was terminated by filtration over GF/B filterplates on a FilterMate harvester (PerkinElmer). The filters were dried and 25 μL Microscint 20 (PerkinElmer) was added to each filter. After ≥3 hours of extraction the bound radioactivity was determined in a Wallac Microbeta Trilux 1450 counter (PerkinElmer, MA, USA).

ERK1/2 phosphorylation assay

The assay was performed using the AlphaScreen SureFire Phospho-ERK1/2 kit

(PerkinElmer, MA, USA), following the kit protocol. Briefly, a 96-well cell culture plate was coated with poly-D-lysine and HEK cells stably expressing human HCA_2 were seeded at 50,000 cells/well in 200 μL DMEM supplemented with 10% newborn bovine serum, 0.4 mg/mL G418, 50 IU/mL penicillin and 50 μg/mL streptomycin. After overnight incubation the cells were serum starved for 4 h in the same medium lacking the serum, and then the medium was replaced by 90 μL prewarmed PBS and incubated for an additional 30 minutes. Increasing concentrations of the test compounds were diluted in prewarmed PBS and 10 μL was added per well for stimulation. After 5 minutes the stimulation solution was removed from the plates, the wells were washed once in ice-cold PBS and 100 μL lysis buffer was added per well. After 15 minutes of incubation and shaking at room temperature, the lysates were mixed by pipetting and 4 μL was transferred to a 384-well OptiPlate (PerkinElmer, MA, USA). The reaction mix was prepared according to the kit protocol (60 μL reaction buffer and 10 μL activation buffer with 1 μL of the donor and acceptor beads each) and 7 μL mix was added to each proxyplate well. After 2 h the plate was read on an EnVision multilabel plate reader (PerkinElmer, MA, USA).

Animals

Twelve week old female C57BL/6 mice were fed a regular cholesterol-free chow diet containing 4.3% (w/w) fat (RM3, Special Diet Services, Witham, UK). Mice received niacin, or HCA_2 partial agonists LUF6281 and LUF6283 (400 mg/kg/day in 50% (v/v) DMSO in PBS) once a day for 4 weeks via oral gavage. After euthanization, mice were bled via orbital exsanguination and perfused in situ through the left cardiac ventricle with ice-cold PBS (pH 7.4) for 20 minutes. Liver and fat were dissected and snap-frozen in liquid nitrogen. Animal care and procedures were performed in accordance with the national guidelines for animal experimentation. All protocols were approved by the Ethics Committee for Animal Experiments of Leiden University.

Measurement of skin flushing

Cutaneous flushing in C57BL/6 mice was assessed by monitoring the change of the skin temperature at the mouse paw location. Temperature measurements were recorded using a non contact infrared thermometer (Pro Exotics PE-1 Infrared Temp Gun, Littleton, USA). The probe was held at a distance of 1 to 2 mm from the metacarpal pad of the mouse paw, and temperature readings were taken from a circular area approximately 3 mm in diameter. Animals were habituated to handling and to the infrared probe before use. Skin temperature was initially recorded from the abdominal area, tail, ear, and paw, after which it was determined that mouse paw skin temperature yielded the most reliable and consistent results. During the experiment, the animals were dosed with niacin or partial agonists LUF6281 and LUF6283 (400 mg/kg/day) via oral gavage (10:00-11:00 AM), and the paw temperature was measured every 10 minutes for a period of 60 minutes in total. Three readings from the center area of mouse paw were recorded routinely for each time point. Baseline paw temperature was recorded right before animals were dosed. All the administration was performed in conscious mice to avoid the interference of the anesthetics on skin temperature.

Plasma lipid analysis

The distribution of cholesterol over different lipoproteins in plasma was determined by fast protein liquid chromatography (FPLC) through a Superose 6 column (3.2 x 30 mm; Smart-System, Pharmacia, Uppsala, Sweden). Cholesterol content of the lipoprotein fractions was measured using the enzymatic colorimetric assay (Roche Diagnostics, Mannheim, Germany).

Quantitative real-time PCR

Total RNA was isolated using acid guanidinium thiocyanate (GTC)-phenol-chloroform extraction. Briefly, 500 μL of GTC solution (4 M guanidine isothiocyanate, 25 mM sodium citrate, 0.5% N-lauroylsarcosine) was added to each sample, followed by acid phenol:chloroform extraction. RNA in the aqueous phase was precipitated with isopropanol. The quantity and purity of isolated RNA were examined using an ND-1000 Spectrophotometer (Nanodrop, Wilmington, DE, USA). One microgram of RNA from each sample was converted into cDNA by reverse transcription with RevertAid™ M-MuLV Reverse Transcriptase (Promega, Madison, WI, USA). Negative controls without addition of reverse transcriptase were prepared for each sample. Quantitative real-time PCR was carried out using ABI Prism 7700 Sequence Detection system (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. 36B4, beta-actin, and GAPDH were used as internal housekeeping genes. The gene-specific primer sequences used are listed in Table 1. Amplification curves were analyzed using 7500 Fast System SDS software V1.4 (Applied Biosystems, Foster City, CA, USA).

Table 1. Primers used for quantitative real-time PCR analysis

Gene	Forward primer	Reverse Primer
36B4	GGACCCGAGAAGACCTCCTT	GCACATCACTCAGAATTTCAATGG
beta-actin	AACCGTGAAAAGATGACCCAGAT	CACAGCCTGGATGGCTACGTA
GAPDH	TCCATGACAACTTTGGCATTG	TCACGCCACAGCTTTCCA
ATGL	TGCCCTCAGGACAGCTCC	TTGAACTGGATGCTGGTGTTG
HSL	CTGACAATAAAGGACTTGAGCAACTC	AGGCCGCAGAAAAAAGTTGAC
APOB	ATGTCATAATTGCCATAGATAGTGCCA	TCGCGTATGTCTCAAGTTGAGAG
MTP	AGCTTTGTCACCGCTGTGC	TCCTGCTATGGTTTGTTGGAAGT

Statistical analysis

Analysis of the in vitro studies was performed using Prism 5.0 software (GraphPad Software Inc., La Jolla, USA). Nonlinear regression was used to determine IC_{50} values from competition binding curves. The Cheng-Prusoff equation was then applied to calculate K_i values [21]. [^{35}S]-GTPγS and pERK curves were analysed by nonlinear regression to obtain EC_{50} values. For the in vivo studies, data were analyzed by T-test or one way ANOVA with Student-Newman-Keuls post test (Instat GraphPad software, San Diego, USA) were appropriate. Statistical significance was defined as $p<0.05$. Data are expressed as means+SEM.

Results

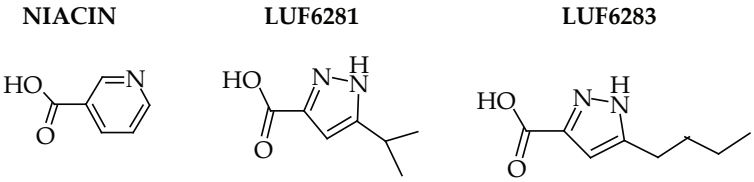

Figure 1. Chemical structures of niacin, LUF6281, and LUF6283.

The chemical structures of niacin and the HCA_2 partial agonists LUF6281 and LUF6283 are shown in Figure 1. The affinity of the compounds for HCA_2 was determined by a competitive binding assay using radiolabeled nicotinic acid and a HEK293T cell line stably expressing the human HCA_2 nicotinic acid receptor (Figure 2). The K_i values determined for LUF6281 and LUF6283 were 3.1 μM and 0.55 μM, respectively (Table 2).

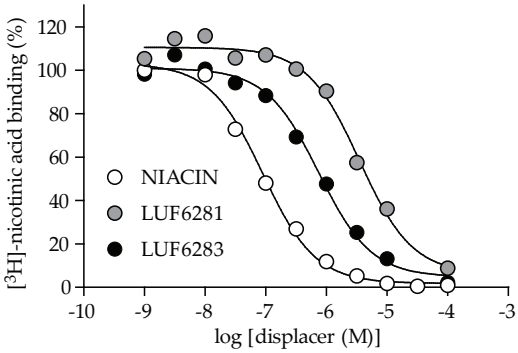

Figure 2. Competitive radioligand binding assay using 20 nM [^{3}H]-nicotinic acid revealing the relative affinities of niacin, LUF6281 and LUF6283. The assay was performed on HEK293T-HCA_2 membranes (50 μg/tube). A representative experiment is shown (of N=3).

Table 2. In vitro biochemical characterization of the HCA_2 agonists niacin, LUF6281, and LUF6283. Values are means±SEM (N≥3).

	K_i (μM)	EC_{50}-[^{35}S]-GTPγS (μM)	EC_{50}-pERK1/2 (μM)	EC_{50} ratio GTPγS/pERK1/2
Niacin	0.04 ± 0.02	0.41 ± 0.11	0.02 ± 0.004	21
LUF6281	3.1 ± 0.5	8.60 ± 1.00	1.37 ± 0.31	6
LUF6283	0.55 ± 0.01	3.10 ± 0.13	0.32 ± 0.06	10

As functional readout the potencies and intrinsic efficacies of niacin, LUF6281, and LUF6283 were measured by their ability to stimulate [^{35}S]-GTPγS binding. The results show that LUF6281 and LUF6283 are partial agonists compared to niacin (100%), with intrinsic efficacies of 55±4% and 76±3%, respectively (N=3). The rank order of their potency was niacin > LUF6283 > LUF6281, with EC_{50} values of 0.41, 3.1 and 8.6 μM, respectively (Figure 3; Table 2).

The second functional assay monitored ERK1/2 phosphorylation upon HCA_2 activation by the different compounds. All compounds appeared to be high efficacy full agonists (Figure 4; Table 2). The EC_{50} values obtained here were 20 nM for niacin, 1.4 μM for

LUF6281 and 0.32 μM for LUF6283. Thus, all compounds seemed to be more potent in the pERK1/2 assay than in the [^{35}S]-GTPγS assay. Importantly, this difference was much more pronounced for niacin (21-fold) than for LUF6283 (10-fold) and LUF6281 (6-fold) (Table 2). To examine the vasodilatory effects of these compounds in vivo, we used normolipidemic C57BL/6 mice to assess the cutaneous flushing. Normal paw skin temperature of C57BL/6 mice is approximately 26.4°C (n=30). Flushing was measured as absolute increase in mouse paw skin temperature. As anticipated, niacin treatment induced a strong increase in skin temperature (+3°C; P<0.001). However, neither of the partial agonists displayed a significant temperature rise. At 20 minutes after compound administration, LUF6281 and LUF6283 induced a temperature increase of maximally 0.6°C (n = 10 per group), which was significantly lower than the temperature rise observed in the niacin group (P<0.001; Figure 5) and not different from the temperature rise induced by mouse handling alone. Both of the HCA_2 partial agonists thus avoided the unwanted flushing side effect seen upon niacin exposure in mice.

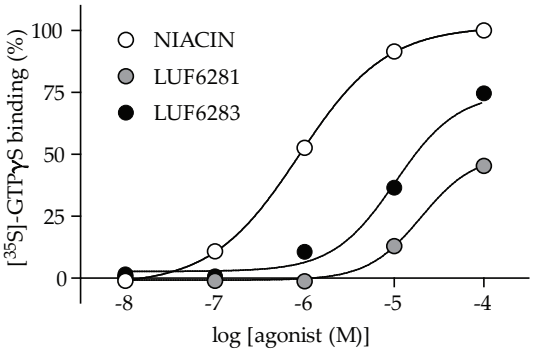

Figure 3. Concentration-response curves of niacin, LUF6281 and LUF6283 in a [^{35}S]-GTPγS binding assay, showing the relative potencies and intrinsic efficacies. Niacin is a full agonist, whereas LUF6281 and LUF6283 are partial agonists in this assay. The assay was performed on HEK293T-HCA_2 membranes (5 μg/tube). A representative experiment is shown (of N=3).

To evaluate the lipid lowering potential of the LUF compounds, we tested their effect on plasma lipid levels. Although treatment with niacin or the LUF compounds for 4 weeks did not alter the plasma total cholesterol or triglyceride concentrations (data not shown), separation of plasma lipoproteins by FPLC in combination with analysis of the lipid content across the FPLC fractions showed that both niacin and the two partial agonists

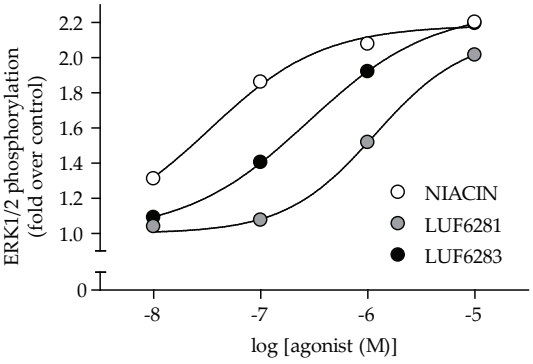

Figure 4. Concentration-response curves of niacin, LUF6281 and LUF6283 in an ERK1/2 phosphorylation assay, showing the relative potencies and intrinsic efficacies. All ligands are full agonists in this assay. The assay was performed on attached HEK293T-HCA_2 cells. A representative experiment is shown (of N=3-5).

greatly reduced plasma VLDL-triglyceride concentrations in C57BL/6 mice (Figure 6).

It is well established that niacin lowers plasma triglycerides through its anti-lipolytic action in adipocytes. In accordance, in white adipose tissue of niacin-treated mice we detected a marked decrease in the relative mRNA expression level of key lipolytic enzymes adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL; Figure 7). Strikingly, no effect of the partial agonists on adipose tissue ATGL/HSL expression was noted. In contrast, in liver, LUF6281 and LUF6283 significantly reduced (-50%; $P<0.05$) the expression of apolipoprotein B (APOB), the essential protein moiety of triglyceride-rich VLDL/LDL particles, while niacin did not (Figure 8). None of the treatments significantly changed the expression level of microsomal triacylglycerol transfer protein (MTP) in livers of C57BL/6 mice, suggesting that the loading of APOB with lipids was not affected. Combined, these findings suggest that the partial agonists lower plasma VLDL-triglycerides levels by interfering with hepatic VLDL production rather than inhibiting adipocyte lipolysis.

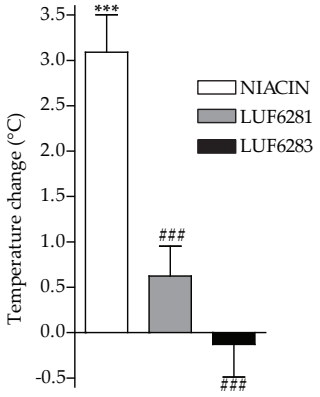

Figure 5. Mouse flushing after administration of niacin, LUF6281, and LUF6283. The cutaneous vasodilation was determined by change in paw skin temperature in C57BL/6 mice. Mice received niacin, LUF6281, or LUF6283 (400 mg/kg/day) via oral gavage. Data are expressed as the change of skin temperature at 20 min after compound administration (n=10 per group). ***$P<0.001$ vs untreated mice; ### $P<0.001$ vs niacin-treated mice.

Discussion and conclusions

Although niacin can effectively lower lipid levels through inhibition of adipocyte lipolysis, its clinical use has been restricted by its off target cutaneous flushing effect that is mediated by HCA_2 located in skin Langerhans cells and keratinocytes [12, 22]. To overcome this problem, a variety of niacin derivates have been developed in the past that act as partial or biased agonists for HCA_2 [18-19, 23]. To date, the most promising lead compound has been the pyrazole MK-0354 which was shown to stimulate the G protein pathway associated with anti-lipolysis in adipocytes without inducing parallel ERK1/2 phosphorylation, an in vitro measure for flushing [23]. Also in pre-clinical mouse studies MK-0354 showed promising results as no vasodilatation (flushing) was observed while the anti-lipolytic activity was retained. Unfortunately, in clinical trials this compound eventually failed since it did not provide the beneficial effect of niacin on plasma lipid levels [17]. Although the clinical failure of MK-0354 argues against a high potential for this class of compounds in general, our current studies indicate that there is still hope for pyrazoles. More specifically, here we present data on the potential of two novel partial HCA_2 agonists of the pyrazole class to lower plasma VLDL-triglyceride levels without

causing the off target flushing response.

Our in vitro studies suggest that niacin, LUF6281, and LUF6283 may all have a certain bias, since these compounds all show a higher potency for ERK1/2 phosphorylation than for G protein activation. Furthermore, LUF6281 and LUF6283 were both partial agonists in the [^{35}S]-GTPγS assay but high efficacy full agonists in the ERK1/2 phosphorylation assay. The fold difference in potency was dependent on the compound; niacin was 21-fold more potent for ERK phosphorylation, while LUF6283 and LUF6281 were respectively 10-fold and only 6-fold more potent. The high potency of niacin for activation of the MAP kinase pathway may explain why this compound causes flushing so effectively. Previous findings of Walters et al. have suggested an important role for an additional pathway, i.e. beta-arrestin dependent signaling, in ERK phosphorylation and the flushing response

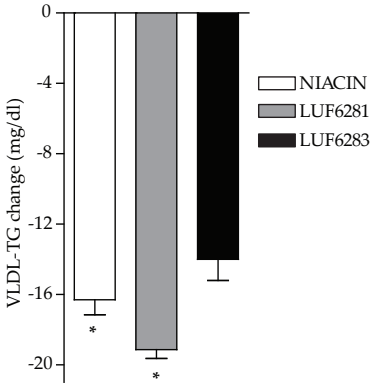

Figure 6. Effect of niacin, LUF6281, and LUF6283 on the plasma VLDL-triglyceride concentration in C57BL/6 mice. Mice were fed a regular chow diet and received either niacin or HCA_2 partial agonists LUF6281 and LUF6283 (400 mg/kg/day) once a day for 4 weeks. Plasma lipoproteins were separated by FPLC and the triglyceride level was measured in each fraction. Fractions 2 to 7 represented VLDL. Values are means+SEM (n=3/4 per group). *P<0.05 vs untreated mice.

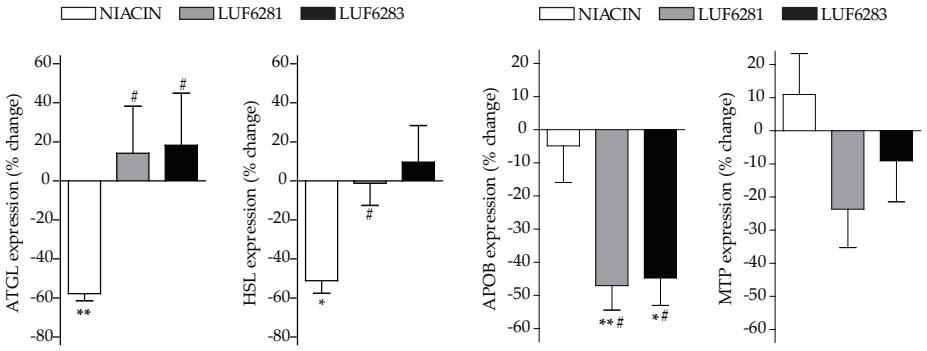

Figure 7. Effect of niacin, LUF6281, and LUF6283 on relative gene expression levels of adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL) in white adipose tissue of C57BL/6 mice. Data are presented as percent change relative to the untreated group. Values are means+SEM (n=5/6 per group). *P<0.05 ** P<0.01 vs untreated mice; # P<0.05 vs niacin-treated mice.

Figure 8. Effect of niacin, LUF6281, and LUF6283 on hepatic relative gene expression levels of apolipoprotein B (APOB) and microsomal triacylglycerol transfer protein (MTP) in C57BL/6 mice. Data are presented as percent change relative to the untreated group. Values are means+SEM (n=5/6 per group). *P<0.05 ** P<0.01 vs untreated mice; # P<0.05 vs niacin-treated mice.

associated with niacin treatment [24]. Since we do not possess data on the effect of our compounds on beta-arrestin membrane recruitment, we cannot draw a firm conclusion whether our compounds are biased partial agonists. However, since all three compounds

in the current study are more potent in the ERK-phosphorylation assay (lower EC_{50} values), we do not anticipate that biased agonism is the reason for their negligible flushing effect. Although, unlike MK-0354, our pyrazole compounds are still active in the ERK1/2 assay, we hypothesized that their relatively low ratio of GTPγS over ERK1/2 might still attenuate the flushing response. Indeed, our in vivo findings in C57BL/6 mice confirm that the pyrazoles do not provoke a similar flushing response as niacin.

Both pyrazoles however induced a similar level of VLDL-triglyceride lowering as observed with niacin treatment, suggesting that they do also exhibit the anti-lipolytic activity of niacin. Strikingly, niacin treatment decreased the relative expression level of the lipolytic genes ATGL and HSL in adipose tissue, while the two LUF compounds did not. From these combined findings it seems that the novel partial agonists – in contrast to niacin – actually do not execute their VLDL-triglyceride lowering action by modulating adipocyte lipolysis. Our further gene expression analysis suggests that the compounds rather lower plasma triglyceride levels by inhibiting VLDL production, since they decrease the hepatic relative expression level of APOB. The assembly and secretion pathway of VLDL in the liver involves the transfer of lipid by MTP to APOB during translation and then the fusion of APOB-containing precursor particles with triglyceride droplets to generate mature VLDL particles [25-26]. APOB is thus essential for proper assembly and secretion of APOB-containing lipoproteins [27] and several novel lipid lowering therapies are therefore based on decreasing APOB transcription and mRNA stability by antisense oligonucleotides [28-29]. As our new HCA_2 partial agonists target another metabolic pathway, i.e. hepatic VLDL production, as compared to both niacin and MK-0354 (inhibition of adipocyte lipolysis), we anticipate that they may still also effectively achieve lipid lowering in the human situation. However, their efficacy in the clinical setting remains to be validated.

In conclusion, the current study identifies the two HCA_2 partial agonists LUF6281 and LUF6283 as promising drug candidates to achieve the beneficial lipid lowering effect of niacin without producing the unwanted flushing side effect.

Acknowledgements

This work was supported by Top Institute Pharma (Grant D1-105 to C.C.B. and A.P.IJ.; Grant T2-110 to Z.L., R.J.V.D.S., T.J.C.V.B., and M.H.) and the Netherlands Heart Foundation (Grant 2008T070 to M.H.).

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