Differential effects of 5-HT_3 receptor antagonist on lipid profile in spontaneously hypertensive rat and chromosome 8 congenic strain

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Abstract

OBJECTIVES: Ondansetron is an antagonist of 5-HT_3 receptors mostly used as an antiemetic yet known to modulate metabolism and appetite. We tested the metabolic effects of ondansetron in newly derived congenic rat strain, carrying limited chromosome 8 regions of (PD) Brown Norway (BN) and polydactylous (PD) strain origins (including variant serotonin receptor *Htr3b* gene) within the genomic background of highly inbred model of metabolic syndrome, the spontaneously hypertensive rat (SHR).

METHODS: Adult, standard diet-fed male rats of SHR and the congenic SHR.(PD/BN)8 strains received ondansetron (2mg/kg body weight/day) or vehicle (n=6/strain/treatment) via oral gavage for 14 days while we followed their metabolic and morphometric profiles including glucose tolerance and triacylgycerol and cholesterol concentrations in 20 lipoprotein fractions.

RESULTS: We fine-mapped the chromosome 8 differential segment in the new SHR.(PD/BN)8 congenic strain: it comprises BN-derived region together with an adjacent 422kb stretch of PD origin. The SHR.(PD/BN)8 rats were heavier than SHR, the fasting glucose was significantly higher in ondansetron-treated congenic than in SHR (*post-hoc* Tukey's HSD p=0.02). Compared to SHR, ondansetron induced significantly more robust increases of cholesterol and triacylglycerol concentrations in total, chylomicron, VLDL and HDL particles in the SHR.(PD/BN)8 congenic strain.

CONCLUSION: We established new congenic model with distinct pharmacogenetic profile related to metabolic effects of ondansetron, facilitating thus the search for responsible genetic variants within the limited genomic region demarcated by the differential segment.

ORIGINAL ARTICLE

Abbreviations:

5-HT	- 5-hydroxytryptamine
AUC	- Area under the curve
BN	- Brown Norway rat
C	- Cholesterol
EFP	- Epididymal fat pad
HDL	- High-density lipoprotein
HPLC	- High performance liquid chromatography
LDL	- Low-density lipoprotein
OGTT	- Oral glucose tolerance test
OND	- Ondansetron
PD	- Polydactylous rat
RFP	- Retroperitoneal fat pad
RGD	- Rat Genome Database
SHR	- Spontaneously hypertensive rat
TG	- Triacylglycerol
VLDL	- Very low-density lipoprotein

INTRODUCTION

Ondansetron is a competitive antagonist of the 5-hydroxytryptamine (5-HT)$_3$ receptor with structural similarity to 5-HT, clinically used mostly in treatment of chemotherapy-induced and post-operative nausea and vomiting (Machu 2011). However, antiflogistic (Liu *et al.* 2012), antiproliferative effects (Prada *et al.* 2012) of ondansetron have been documented as well as its modulatory actions on food intake (Hayes et al., 2004) and metabolism (Carvalho *et al.* 2002; Carvalho *et al.* 2005; Ozmen & Kufrevioglu 2004). The response to ondansetron varies and part of this variation is presumed to depend on genetic polymorphisms. In spite of the appreciation of importance of pharmacogenetic aspects of ondansetrone action, this area is only modestly developed (Ho and Gan 2006). One of the potentially important genetic factors in this respect is the genetic variation directly in the 5-HT$_3$ receptor. The genes coding for 5-HT$_3$ receptor subunits *Htr3a* and *Htr3b* are co-localized on human chromosome 11q23 and in syntenic region of rat chromosome 8. We have previously identified substitution mutation in *Htr3b* gene in the polydactylous rat strain (Liska *et al.* 2009), a model of metabolic syndrome (Seda *et al.* 2005a; Sedova *et al.* 2000). In this study, we contrasted the metabolic effects of ondansetron in spontaneously hypertensive rat vs. a newly derived congenic strain, carrying a limited segment of rat chromosome 8 (including *Htr3a* and *Htr3b* genes) of polydactylous and Brown Norway rat origins.

METHODS

Rat strains

The spontaneously hypertensive rat (SHR/OlaIpcv, SHR hereafter, Rat Genome Database (RGD) ID: 631848) was originally derived by recurrent selective breeding of Wistar rats in early 60s of the 20th century in Japan. The SHR colony in Prague was obtained from the National Institutes of Health (USA) over 35 years ago and since then it has been maintained by brother x sister mating. The polydactylous rat strain (PD/Cub, PD hereafter, RGD ID no. 728161) is a highly inbred strain showing metabolic syndrome attributes (Seda *et al.* 2005a; Sedova *et al.* 2000), kept since 1969 at the Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University in Prague. In this study we have established new congenic strain combining the genomic information of two genetic models of metabolic syndrome, SHR and PD, together with that of Brown Norway (BN), using marker-assisted backcross breeding. After verifying the congenicity of the new congenic strain by a genome-wide polymorphic marker scan, we defined the extent of the PD- and BN-derived regions by genotyping polymorphic microsatellite markers (see *DNA extraction, genotyping*).

Experimental protocol

All experiments within our project were performed in conformity with the Animal Protection Law of the Czech Republic (311/1997), which is in compliance with the European Community Council recommendations for the use of laboratory animals 86/609/ECC and was approved by the Ethical committee of the First Faculty of Medicine. Adult rat males were held under temperature and humidity controlled conditions on 12h/12h light-dark cycle. At all times, the animals had free access to food (standard chow) and water. Male SHR (n=12) and SHR.(PD/BN)8 (n=12) rats were fed standard laboratory chow *ad libitum*. At the age of 16 weeks, the rats were randomly split to experimental (n=6/strain) and control (n=6/strain) groups. All groups continued to be fed standard diet, and at the same time, the experimental group was administered ondansetron (OND, 2 mg/kg/day via oral gavage) for 14 days while the control group received only vehicle via oral gavage. The food consumption, total body weight and non-fasting glycaemia were followed daily during this period. The rats were sacrificed in postprandial state and the weights of heart, liver, kidneys, adrenals, soleus muscle, epididymal and retroperitoneal fat pads were determined.

DNA extraction, genotyping

The polymerase chain reaction (PCR) was used for genotyping markers polymorphic between progenitor strains. We tested the DNA of congenic SHR.(PD/BN)8 strain and progenitor strains SHR, PD and BN. The rat DNA was isolated by a modified phenol extraction method from tail incisions samples. Nucleotide sequences of primers were obtained from public databases (RGD, http://rgd.mcw.edu/, The Welcome Trust Centre for Human Genetics, http://www.well.ox.ac.uk/ or Whitehead Institute/MIT Center for Genome Research, http://www-genome.wi.mit.edu/). The PCR products were separated on polyacrylamide (7–10%)

gels, detected in UV light after ethidium-bromide staining using Syngene G:Box.

Metabolic measurements

The oral glucose tolerance test (OGTT) was performed after overnight fasting. The blood samples for the glycemia determination (Ascensia Elite Blood Glucose Meter; Bayer HealthCare, Mishawaka, IN, validated by Institute of Clinical Biochemistry and Laboratory Diagnostics of the First Faculty of Medicine) were drawn from the tail vein at intervals of 0, 30, 60, 90, 120 and 180 minutes after the intragastric glucose bolus administration to conscious rats (3g/kg body weight, 30% aqueous solution). The lipid profile including cholesterol and triacylglycerol concentration in 20 lipoprotein fractions and glycerol level was assessed by high performance liquid chromatography method (HPLC) as described previously (Krupkova *et al.* 2009, Sedova *et al.* 2007, Usui *et al.* 2002).

RESULTS

Genomic characteristics of the differential segment in the new SHR.(PD/BN)8 congenic strain

Our genotyping scan of polymorphic microsatellite markers revealed the extent of the chromosome 8 differential segments of PD and BN origin in the SHR.(PD/BN)8 congenic strain. While the BN-derived segment spans about 40 Mb, the adjacent stretch of PD origin (including the previously identified variant H364R in *Htr3b* gene) is only about 422 kb long (Table 1). Several total genome scans conducted throughout the derivation of SHR.(PD/BN)8 strain excluded presence of other non-SHR alleles than those present on chromosome 8, confirming the congenicity of the new strain. The BN- and PD-derived segments on chromosome 8 thus represent the only genomic differences between SHR and SHR.(PD/BN)8 strains.

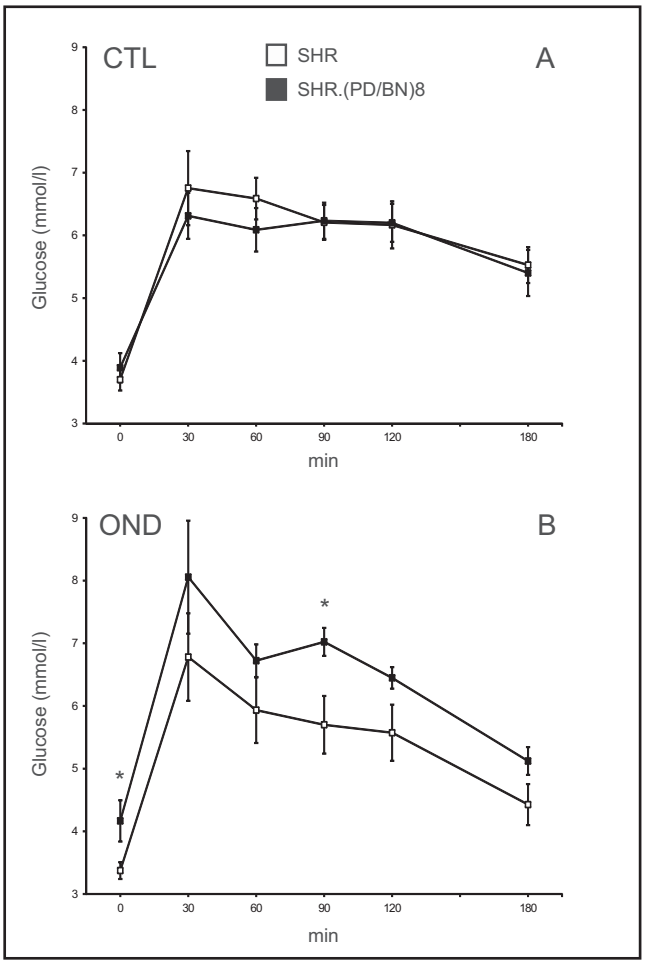

Fig. 1. The course of glycaemic curves in control (CTL, panel A) and ondansetron-treated (OND, panel B) SHR (open symbols) vs. SHR.(PD/BN) (closed symbols) male rats. Within the graphs, the significance levels of strain comparison (SHR vs. SHR.(PD/BN)) by post-hoc Tukey's honest significance difference test of the two-way ANOVA with STRAIN and OND as major factors are indicated as follows: *…p<0.05; **…p<0.01; ***…p<0.001.

Tab. 1. The differential segment in SHR.(PD/BN)8 congenic strain.

Marker	Start (bp)	End (bp)	Origin in SHR.(PD/BN)8
D8Rat53	19815642	19815800	SHR
D8Mit5	32203394	32203499	SHR
D8Rat85	46113659	46113959	SHR
D8Rat41	50354908	50355063	SHR
D1Rat405	51967007	51967244	SHR
21652	52033239	52033378	PD
D8Got72	52065613	52065790	PD
21703	52084614	52084811	PD
21783	52164280	52164394	PD
21807	52188109	52188258	PD
Htr3b* (H364R)		52198655	PD
21829	52209789	52209946	BN
22019	52389888	52390074	BN
D8Rat94	52479599	52479702	BN
D8Rat44	53106706	53107102	BN
D8Rat149	58692901	58693265	BN
D8Mgh6	68789820	68790232	BN
D8Rat26	79347527	79347688	BN
D8Rat75	89558869	89558994	BN
D8Rat19	98451122	98451660	SHR
D8Rat65	109862358	109862585	SHR
D8Rat72	118725143	118725315	SHR

The extent of the differential segment of chromosome 8 in SHR.(PD/BN)8 congenic strain. The microsatellite markers, their genomic positions according to Rat Genome Sequencing Consortium v3.4 reference genome assembly and their origin in the SHR.(PD/BN)8 are shown. * the *Htr3b* (H364R) substitution variant was assessed by allele-specific polymerase chain reaction.

Tab. 2. Morphometric comparison of control and ondansetron-treated SHR vs. SHR.(PD/BN)8 rats.

Trait	CONTROL		ONDANSETRON	
	SHR (n=6)	SHR.(PD/BN)8 (n=6)	SHR (n=6)	SHR.(PD/BN)8 (n=6)
Body weight, g	270±22	306±17[b]	287±12	307±22
Organ weights				
Liver, g/100g b.wt.	3.06±0.07	2.86±0.29[a]	2.78±0.05‡	3.02±0.20[b]
Heart, g/100g b.wt.	0.41±0.02	0.36±0.02[b]	0.38±0.02*	0.37±0.05
Kidney, g/100g b.wt.	0.71±0.07	0.76±0.05	0.65±0.02*	0.70±0.02
Adrenals, mg/100g b.wt.	14.6±0.7	16.9±0.4[c]	13.7±0.7	15.0±0.5[a]†
EFP wt., g/100g b.wt.	1.10±0.10	1.08±0.17	0.87±0.07†	0.82±0.05†
RFP wt., g/100g b.wt.	1.18±0.10	1.11±0.32	1.17±0.17	0.97±0.12

The significance levels are indicated as follows: a,b,c…p<0.05, 0.01 and 0.001, respectively for differences between SHR and SHR.(PD/BN)8 under conditions of a single diet; *, †, ‡… p<0.05, 0.01 and 0.001, respectively, for OND effect within individual strain. Values are shown as mean ± S.D..

Tab. 3. Major triacylglycerol, cholesterol subfractions and free glycerol comparison between control and ondansetron-treated SHR vs. SHR.(PD/BN)8 rats.

Trait (mg/dl)	CONTROL		ONDANSETRON	
	SHR (n=6)	SHR.(PD/BN)8 (n=6)	SHR (n=6)	SHR.(PD/BN)8 (n=6)
Triacylglycerol (TG)				
Total TG	20.33±1.71	22.18±3.50[a]	25.03±3.97	32.43±2.69‡[b]
Chylomicron TG	0.51±0.24	0.47±0.27	0.52±0.20	1.34±0.64†[b]
VLDL-TG	5.48±0.74	5.89±2.40	7.19±2.30	11.04±2.33‡[b]
LDL-TG	8.09±0.66	9.58±0.66[a]	10.90±1.69‡	11.18±1.10*
HDL-TG	6.25±1.27	6.25±0.54	6.42±0.64	8.87±0.64‡[c]
Cholesterol (C)				
Total C	40.92±2.18	35.94±1.74[c]	42.05±2.30	46.72±1.62‡[b]
Chylomicron C	0.23±0.07	0.25±0.10	0.15±0.02	1.06±0.20‡[c]
VLDL-C	1.04±0.15	1.02±0.34	1.15±0.27	2.20±0.49‡[c]
LDL-C	8.84±0.86	7.24±0.93[a]	9.81±1.40	9.74±0.74†
HDL-C	30.82±1.52	27.44±0.91[b]	30.95±1.74	33.74±1.86†[b]
Glycerol	4.04±0.86	5.95±1.20[b]	4.96±0.93	5.93±1.13

Data are shown as mean ± S.D. The significance levels are indicated as follows: a,b,c… p < 0.05, 0.01 and 0.001, respectively for differences between SHR and SHR.(PD/BN)8 under conditions of a single diet; *, †, ‡… p < 0.05, 0.01 and 0.001, respectively, for OND effect within individual strain.

Morphometry and glucose tolerance

The control males of SHR.(PD/BN)8 were significantly heavier than the control SHR rats, however, ondansetron administration erased this difference (Table 2). Ondansetron induced significant reduction of visceral, but not retroperitoneal fat weight in both strains. Opposite trends were observed for relative liver weight (reflected by significant STRAIN * OND interaction – Table 4).

The control groups of both strains did not differ in any measure of glucose tolerance (Figure 1A). However, the OND-treated SHR.(PD/BN)8 congenic displayed significantly higher fasting glycemia (Figure 1B) and impaired glucose tolerance measured by area under the glycemic curve (SHR.(PD/BN)8: 1910±99 mmol/l/180min vs. SHR: 1594±99 mmol/l/180min, *post-hoc* Tukey's HSD p=0.023) when compared to SHR.

Detailed lipid profile

Both control and OND-treated SHR.(PD/BN)8 showed higher concentration of total triacylglycerols (TG) compared to the respective SHR groups, moreover, OND induced significant total TG rise only in the congenic strain (Table 3). While in control rats the difference in total TG was driven exclusively by the higher TG content of small and very small LDL particles (Figure 2A), ondansetron triggered increases in TG across the whole lipoprotein spectrum in SHR.(PD/BN)8 and only in LDL of SHR (Table 3, Figure 2B). Total cholesterol (C) was significantly higher in SHR compared to SHR.(PD/BN)8 in control conditions, mostly due to differences in C content of LDL and HDL (Table 3). However, OND induced massive rise of C concentrations across all fractions exclusively in SHR.(PD/BN)8, resulting in 30% increase in total C vs. no change observed in SHR rats (Table 3, Figures 3A, 3B).

Statistic analyses

The metabolic and morphometric data were compared by two-way ANOVA with STRAIN and ONDANSETRON as main factors followed by Tukey's honest significance difference test for detailed pair-wise comparisons.

DISCUSSION

In this study, we are reporting pharmacogenetic interaction of ondansetron, antagonist of 5-HT_3 receptors, and a defined portion of rat chromosome 8 genetically isolated in new congenic rat strain. OND administra-

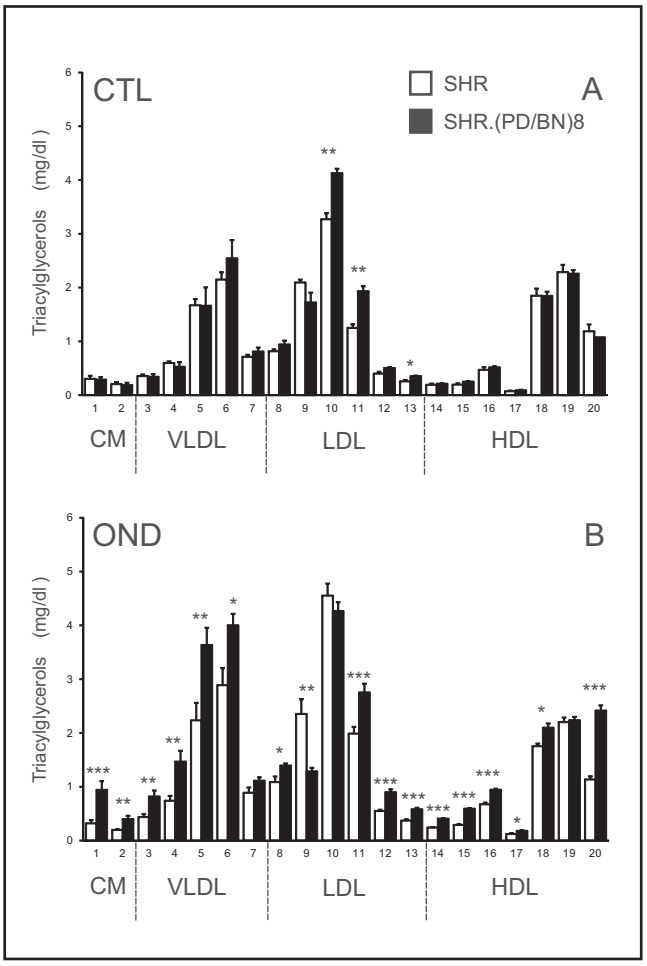

Fig. 2. The triacylglycerol content in 20 lipoprotein subfractions in control (CTL) and ondansetron-treated (OND, panel B) SHR (open symbols) vs. SHR.(PD/BN) (closed symbols) male rats (n=6/strain*treatment). Within the graph, the significance levels of strain comparison (SHR vs. SHR.(PD/BN)) by post-hoc Tukey's honest significance difference test of the two-way ANOVA with STRAIN and OND as major factors are indicated as follows: *p<0.05; **p<0.01; ***p<0.001. The allocation of individual lipoprotein subfractions to major lipoprotein classes is shown in order of particle's decreasing size from left to right.

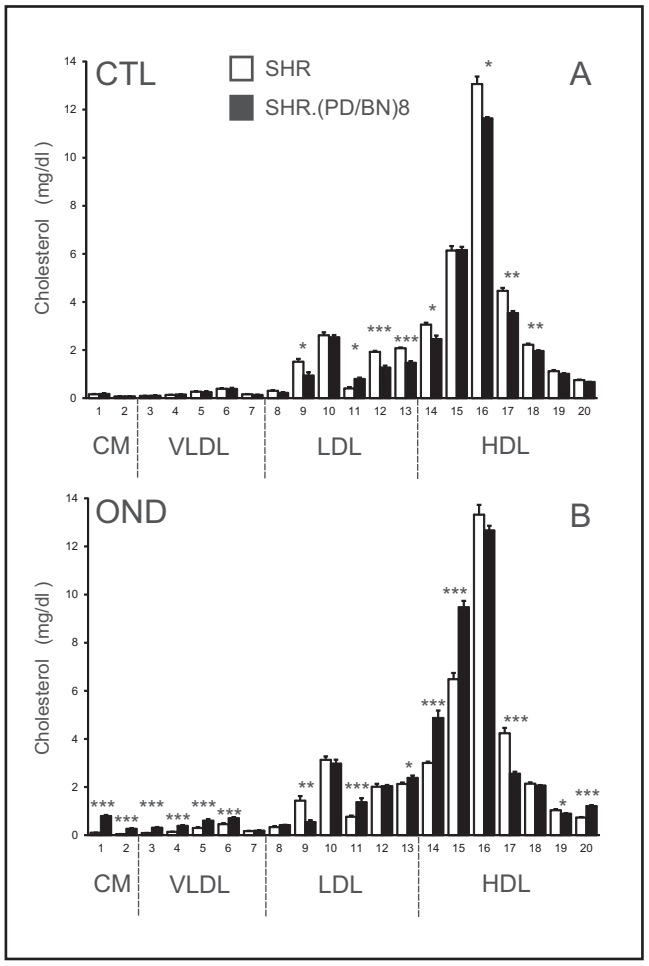

Fig. 3. The cholesterol content in 20 lipoprotein subfractions in control (CTL, panel A) and ondansetron-treated (OND, panel B) SHR (open symbols) vs. SHR.(PD/BN) (closed symbols) male rats (n=6/strain*treatment). Within the graph, the significance levels of strain comparison (SHR vs. SHR.(PD/BN)) by post-hoc Tukey's honest significance difference test of the two-way ANOVA with STRAIN and OND as major factors are indicated as follows: *p<0.05; **p<0.01; ***p<0.001. The allocation of individual lipoprotein subfractions to major lipoprotein classes is shown in order of particle's decreasing size from left to right.

Tab. 4. Two-way analysis of variance (ANOVA) results for morphometric and metabolic profile of SHR vs. SHR.(PD/BN) rats with STRAIN and OND as major factors.

Phenotype	STRAIN	OND	S*OND
Body weight	0.0007	0.27	0.32
Liver, g/100g b.wt.	0.74	0.34	0.0009
Heart, g/100g b.wt.	0.004	0.26	0.07
Kidney, g/100g b.wt.	0.027	0.007	0.89
Adrenals, mg/100g b.wt.	0.0001	0.0015	0.15
EFP wt., g/100g b.wt.	0.50	<0.0001	0.77
RFP wt., g/100g b.wt.	0.07	0.31	0.41
Total C	0.85	<0.0001	<0.0001
Chylomicron C	<0.0001	<0.0001	<0.0001
VLDL-C	0.0014	0.0002	0.0010
LDL-C	0.07	0.0012	0.10
HDL-C	0.65	0.0002	0.0003
Total TG	0.0028	<0.0001	0.047
Chylomicron TG	0.016	0.0086	0.0099
VLDL-TG	0.021	0.0009	0.053
LDL-TG	0.08	0.0003	0.22
HDL-TG	0.0044	0.0017	0.0043
Glycerol	0.0037	0.30	0.28
Glucose (0 min)	0.024	0.91	0.16
Glucose (30 min)	0.56	0.21	0.23
Glucose (60 min)	0.74	0.98	0.15
Glucose (90 min)	0.08	0.70	0.09
Glucose (120 min)	0.27	0.67	0.30
Glucose (180 min)	0.39	0.041	0.22
AUC OGTT (0-180min)	0.0050	0.0040	0.0052

The significance levels of two-way ANOVA's STRAIN, OND and STRAIN*OND (S*OND) factor interactions are shown.

tion slightly impaired the glucose tolerance in the SHR.(PD/BN)8 in comparison to its SHR progenitor strain. The effects of OND on carbohydrate metabolism were reported previously both in experimental models (Carvalho *et al.* 2002; 2005; Mandhane *et al.* 2012) and clinical setting (Patel *et al.* 2011). The potential explanation probably relates to orchestrating role of serotonin in energy balance and blood glucose levels via central nervous system circuitry (Tecott 2007) yet the mechanism remains to be elucidated as many 5-HT receptor subtypes are expressed in the responsible hypothalamic regions. Interestingly, we did not observe any difference in feeding behavior of the OND-treated rats (food intake did not differ significantly throughout the experimental period in neither SHR nor SHR.(PD/BN)8, data not shown), yet clearly shifts have occurred in energy utilization as both OND-treated strains displayed reduced amount of visceral fat and distinct shifts in their lipid profiles. As we administered OND orally, the observed effects are likely to have occurred through modulation of peripheral (intestinal) serotonin stimulation occurring naturally through serotonin release from intestinal enterochromaffin cells following the gastric distension and increased luminal pressure with central effects indirectly mediated via activation of ascending vagal afferent fibers expressing 5-HT_3 receptors (reviewed by Marston *et al.* 2011). Our most striking finding is the substantial rise of triacylglycerol and particularly cholesterol concentrations in the standard-diet fed SHR.(PD/BN)8 in response to OND. Although, to our knowledge, there are no reports concerning the link between OND and cholesterol levels nor dyslipidemia in general, similar effects have been described after use of second-generation antipsychotic drugs with antagonistic effects on 5-HT receptors, e.g. olanzapine (Rummel-Kluge *et al.* 2010). Apart from dyslipidemia induced by the antipsychotics as the secondary effect of the weight gain, there is another, direct causative mechanism more pertinent to the current study (de Leon & Diaz 2007). So far, single-nucleotide polymorphism rs2229416 in acetyl-coenzyme A carboxylase α (*ACACA*) gene has been proposed to be involved in mediation of the abovementioned hyperlipidemic effect, with no clear relevance to genetic content of the differential segment distinguishing the presented rat strains. Dedicated functional genomic studies are necessary to illuminate the network of relations connecting serotonin and lipid homeostasis.

We are aware of several limitations of our study. First, this is a single-dose based study. We have opted for the ondansetron dosage based on our pilot studies (data not shown) and the available information – we have selected relatively higher dosage (2 mg OND/kg/day for 14 days) compared to human clinical practice as comparatively lower bioavailability of ondansetron in rats is documented (Yang and Lee 2008). Second, the congenic strain SHR.(PD/BN)8 may possibly carry several genetic variants distinguishing it from the SHR progenitor as the differential segment (in spite of representing only about 1.5% of the rat genome) comprises dozens of genes. Actually, we have previously shown that mutated *Zbtb16* (*Plzf*) gene (within the PD-derived segment of SHR.(PD/BN)8) in PD coding for promyelocytic leukemia zinc finger is the most likely candidate for pharmacogenetic interactions of dexamethasone (Seda *et al.* 2005b) and retinoic acid (Krupkova *et al.*, 2009). However, this mutation is unlikely to play an important role in this study. Currently, there are 9 genes with annotated evidence (Ingenuity Pathway Analysis, IPA Release Fall 2012, build 172788) of interaction with ondansetron including 5-HT_3 receptor subunits *HTR3A-E*, 5-HT_4 receptor (*HTR4*), solute carrier family 22 (organic cation transporter), member 2 (*SLC22A2*), sigma non-opioid intracellular receptor 1

(*SIGMAR1*) and protein tyrosine phosphatase, receptor type, S (*PTPRS*). Out of those, only *Htr3a* and *Htr3b* are captured in the differential segment of the SHR.(PD/BN)8 congenic strain. Whereas our knowledge of the pharmacogenetic aspects of ondansetron action is incomplete (Ho & Gan 2006), the pharmacogenetic importance of the *HTR3B* variation is well documented in substance abuse (King *et al.* 2012), opioid efficacy (Klepstad *et al.* 2011) or statin-induced myalgia (Ruano *et al.* 2007). While we cannot exclude other polymorphisms present in the segment to mediate the observed distinct reaction to ondansetron treatment, the variant *Htr3b* (H364R) of PD rat (Liska *et al.* 2009) origin remains as the most plausible candidate to be verified in further studies.

In summary, we have established a novel congenic strain showing distinct metabolic response to orally administered ondansetron, creating thus a useful experimental tool for pharmacogenetic and pharmacogenomics analysis of ondansetron's effects on carbohydrate and lipid metabolism.

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