Antagonism of vasopressin V2 receptor improves albuminuria at the early stage of diabetic nephropathy in a mouse model of type 2 diabetes

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Abstract

Aims: Vasopressin is increased in diabetes and was shown to contribute to development of diabetic nephropathy through V2 receptor (V2R) activation in an experimental model of type 1 diabetes. The role of V2R in type 2 diabetes remains undocumented. This study addresses the issue in a mouse model of type 2 diabetes.

Methods: Male obese diabetic *db/db* mice were treated for 12 weeks with a selective V2R antagonist (*SR121463*) and compared to non-treated *db/db* and non-diabetic *db/m* mice. All animals were previously uninephrectomized.

Results: The V2R antagonist did not alter glycaemia or glycosuria in *db/db* mice. It induced a two-fold increase in urine output and a 52% decrease in urine osmolality compared to non-treated *db/db* mice. After four weeks of treatment urinary albumin to creatinine ratio was 50% lower in treated mice compared to non-treated mice, and remained significantly lower until end of experiment. Glomerular filtration rate increased significantly over time in non-treated *db/db* mice but remained stable in treated mice.

Conclusions: This study shows that vasopressin contributes to albuminuria and glomerular hyperfiltration via V2R in a mouse model of type 2 diabetes. It documents causality behind the association of vasopressin with renal disease observed in diabetic patients.

Key words: vasopressin; V2 receptor; antagonist; diabetic nephropathy; type 2 diabetes

1. Introduction

Diabetic nephropathy is a leading cause of end-stage renal disease (ESRD), and a major contributor to the increased mortality observed in subjects with diabetes (Reutens, 2013). An expanding set of data suggests that the vasopressin - hydration axis plays a role in onset and progression of chronic kidney disease (CKD) (Bankir et al., 2013). Increased plasma osmolarity is the main stimulus for vasopressin secretion, which is thus strongly dependent on the hydration status.

Vasopressin is co-secreted in equimolar amount with copeptin, the C-terminal portion of the preprovasopressin peptide. Cross-sectional and prospective studies in the general population have shown associations between plasma copeptin and albuminuria or renal function decline (Meijer et al., 2010; Enhorning et al., 2013; Roussel et al., 2015). High circulating copeptin concentration was also associated with the development and progression of diabetic nephropathy in patients with type 2 diabetes (Boertien et al., 2013; Velho et al., 2013; Hu et al., 2015; Pikkemaat et al., 2015; Zhu et al., 2016), and with prevalence of ESRD in subjects with long-standing type 1 diabetes (Bjornstad et al., 2016; Velho et al., 2016). Experimental data suggests a causal role of vasopressin in renal dysfunction through activation of the V2-receptor (V2R). Indeed, administration of dDAVP, a V2R agonist, to normal rats, induces, besides the well-documented antidiuretic effect, glomerular hyperfiltration and a rise in urinary albumin excretion (UAE) (Bouby et al., 1996; Bardoux et al., 2003a). Similarly, in human subjects, acute administration of dDAVP increases UAE. This effect of dDAVP does not occur in subjects with loss of function mutations in V2R (Bardoux et al., 2003a). In renal diseases, activation of V2R was shown to participate in the progression of renal failure in rats with five-sixth reduction in renal mass (Bouby et al., 1990). In a rat model of type 1 diabetes, vasopressin was shown to contribute to microalbuminuria through its V2R activation (Bardoux et al., 2003b). The role of V2R in kidney complications of type 2 diabetes remains

however so far undocumented.

In the present investigation, we evaluated the contribution of the V2R to the progression of nephropathy in a murine model of type 2 diabetes. The effect of chronic treatment with a selective non-peptide V2R antagonist on renal function parameters was studied in *db/db* mice.

2. Material and methods

2.1. Animals and treatment

All animal procedures were conducted in accordance with the Directive 2010/63/eu of the European Union and were approved by the Animal Care and Ethics Committee of the French Ministry of Research. Reporting of this work complies with ARRIVE guidelines. Male diabetic *db/db* and non-diabetic *db/m* mice bred on a C57BLKS/J background (Janvier Labs - Le Genest Saint Isle, Mayenne, France) were used. Animals were housed in the registered pathogen free facility (A75-06-12) of the Centre de Recherche des Cordeliers with a 12-hour light/dark cycle and had free access to water.

Seven-week old mice of both strains underwent left nephrectomy under pentobarbital anesthesia (Nembutal®, 0.6mg/10g BW), in order to increase the vulnerability of the remnant kidney (Levine et al., 2008). Two weeks after the surgical procedure, animals were housed in metabolic cages (Techniplast, Lyon, France) for two consecutive 24-hour periods for establishing basal values for all parameters including body weight (BW), food and fluid intake, urine flow rate and urine osmolality. Three groups of mice were then studied. Diabetic *db/db* mice treated with the selective V2R antagonist *SR121463* (Sanofi-Recherche, Toulouse, France) for 12 weeks were compared to non-treated diabetic *db/db* mice and to non-diabetic *db/m* mice (n=7 per group). The V2R antagonist was mixed with standard powdered food (A04, Safe, Augy, France) and a small amount of water (0.5 ml/g food). It was administered at 30 mg/kg/day for the first 9 weeks of the protocol and at 45 mg/kg/day for the

remaining 3 weeks. The increase in dose was necessary to maintain the high urine flow rate and low urine concentration in treated animals.

The diabetic *db/db* control and non-diabetic *db/m* mice received the same powdered food/water mix without the drug. In order to ensure the same food intake in all groups and total drug intake in the V2R antagonist treated *db/db* group, all mice were offered a daily amount of standard diet slightly less than their spontaneous intake (3 and 5 g/day for *db/*m and *db/db* mice, respectively).

2.2. Plasma and urinary parameters

Every second week after initiation of treatment, body weight, water and food intake, and urine volume were measured. Data from two 24-hr urine collections were averaged for each animal. The following parameters were measured: osmolality (freezing-point osmometer, Roebling, Germany), creatinine, glucose (Konelab® 20i, Ortho-clinical Diagnostics, Thermo Electron Corporation), sodium (flame photometer 943, Instrumentation Laboratory, Bedford, MA) and albumin (ELISA, Albuwell M, Exocell, USA). Urinary albumin excretion was expressed as albumin/creatinine ratio (ACR). Blood samples were taken by retro-orbital puncture after a 6-hour fasting period for the measurement of plasma creatinine (Konelab® 20i, Orthoclinical Diagnostics, Thermo Electron Corporation), and blood glucose (OneTouch Vita, Lifescan, Switzerland). At the end of the 12^{th} week, mice were anesthetized and sacrificed. The remaining kidney and the heart (minus the auricles) were removed and weighed.

2.3. Blood pressure measurements

Systolic blood pressure was measured in conscious animals at three successive days at weeks 3, 5 and 7 by tail-cuff plesthymography (Blood Pressure System Analysis, Model BP-2000, Visitech System, USA). Data from the last two days were averaged for each mouse.

2.4. Statistical analysis

Data are expressed as mean ± SD unless otherwise specified. Effect of diabetes was assessed by analysis of variance (ANOVA) followed by Fisher *post hoc* test. Effect of the V2R antagonist was assessed by ANOVA for repeated measures followed by Fisher *post hoc* test. Organ weights were compared by Student's *t*-test. $P<0.05$ was considered as statistically significant.

3. Results

Body weight, fasting blood glucose and glycosuria were similar in *SR121463* treated and non-treated diabetic *db/db* mice at baseline, and throughout the study. They were higher in the *db/db* groups than in the non-diabetic *db/m* mice. No difference in systolic blood pressure was observed between groups, 3 weeks after initiation of treatment (Table 1), and blood pressure remained stable in all groups throughout the study (data not shown).

As expected, non-treated *db/db mice* had higher diuresis than *db/m* mice (ANOVA, $p<0.05$) (Figure 1). Blockade of V2R with *SR121463* resulted in a rapid and significant decrease in urine concentration in *db/db* mice. At week 4, urine osmolality was two-fold lower in treated *db/db* than in non-treated *db/db* mice (894 ± 177 *vs.* 1855 ± 858 mosm/kg H_2O, $p<0.05$). Conversely, urine flow rate was higher in treated *db/db* than in non-treated *db/db* mice (10.4 ± 6.1 *vs.* 5.6 ± 3.7 ml/day, $p<0.05$). The diuretic effect of *SR121463* persisted during the 12 weeks of treatment ($p=0.02$) (Figure 1). The V2R antagonist had no effect on osmolar excretion (Table 1) and natriuresis: sodium excretion was 177 ± 40 in *db/m*, 298 ± 37 in non-treated *db/db* and 310 ± 63 µmol/day in treated *db/db* mice after 4 weeks of treatment and 147 ± 28, 291 ± 47, 317 ± 66 µmol/day respectively after 8 weeks.

ACR at baseline was similar in treated and non-treated *db/db* groups. In both groups, it was significantly higher than in the *db/m* group. In non-treated *db/db* mice ACR steadily increased over time up to 179 % of baseline values at week 12. By contrast, in *SR121463* treated *db/db*

mice, ACR initially decreased by roughly 50%, remained stable for the next four weeks and then increased but remained significantly lower ($p<0.02$) than in non-treated *db/db* mice (figure 1).

Compared to *db/m* mice, *db/db* mice showed elevated creatinine clearance rate which indicated significant glomerular hyperfiltration. Creatinine clearance, increased over time in non-treated *db/db* mice (409 ± 95 and 688 ± 96 ml/day, $p<0.05$, at weeks 4 and 8 respectively) but remained stable in treated *db/db* mice (437 ± 185 and 530 ± 226 ml/day, NS) (Figure 2).

At the end of the study, no difference in the absolute or relative weight of the heart was observed among the experimental groups. Absolute kidney weight was similar in non-treated *db/db* and *db/m* mice, while kidney to body weight ratio was lower in *db/db* mice, treated and non-treated *db/db* compared to *db/m* mice due to the increased body weight of *db/db* animals. Kidney weight was reduced ($p<0.05$) in treated *db/db* mice as compared to the non-treated *db/db* group (Table 2).

4. Discussion

We assessed the involvement of vasopressin V2R in the renal dysfunction of an experimental model of type 2 diabetes and diabetic nephropathy, the *db/db* mouse, 7-week-old with unilateral nephrectomy. The main finding of the study is that treatment with the V2R antagonist rapidly induced a decrease in UAE in *db/db* mice compared to non-treated *db/db* mice. These observations are consistent with our previous study in a rodent model of type 1 diabetes, the rat with streptozotocin-induced diabetes (Bardoux et al., 2003b). In *db/db* mice, the V2R antagonist lowered albuminuria and partially prevented hyperfiltration, two main features of incipient diabetic nephropathy. These results support an important role for V2R activation in development of diabetic nephropathy, at least at early stage of the disease.

The mechanism of action of V2R in diabetic nephropathy is not fully elucidated. V2R is active in the collecting duct. Our previous studies in non-diabetic rats suggest that the deleterious effect of vasopressin in the kidney is mainly secondary to the V2R-mediated urine concentrating action of the hormone (Bouby et al., 1990; Bankir et al., 1993). It has been suggested that the activation of V2R in the tubule inhibits the tubuloglomerular feedback as a result of reduction in salt concentration at the macula densa, triggering elevation of intraglomerular capillary pressure, hyperfiltration and glomerular injury (Bankir et al., 2013). Administration of the V2R antagonist attenuated the urine concentrating activity of the kidney without inducing formation of hypo-osmotic urine. Urine osmolality was markedly reduced while urine flow and water intake were increased. Urinary sodium excretion was similar between treated and non-treated *db/db* groups. These data show that the V2R antagonist was a pure and potent aquaretic agent, even in diabetic condition.

Blockade of V2R stimulates pituitary secretion of vasopressin with subsequent V1a receptor (V1aR) activation. Thus, we cannot rule out that the phenotype observed in treated *db/db* mice was, at least in part, indirectly related to an activation of renal, vascular and hepatic V1aR (Cantau et al., 1984; Morita et al., 2001; Taveau et al., 2015). However, lack of increase in blood pressure or glycaemia during V2R antagonist treatment is not in favor of a strong V1aR stimulation.

Renal hypertrophy is also a feature of diabetic nephropathy. In the present study, absolute kidney weight was similar in non-treated *db/db* and non-diabetic *db/m* mice. This could be explained by a masking effect of the compensatory hypertrophy process occurring in the remnant kidney after uninephrectomy, on the hypertrophic effects of diabetes. Similar to our findings, Levine et al found no difference between *db/db* and wild type mice three weeks after uninephrectomy (Levine et al., 2008). However, we observed that the V2R antagonist

treatment slightly attenuated kidney hypertrophy in the uninephrectomized *db/db* mice, consistent with its other renal effects and in agreement with previous studies in rats (Bouby et al., 1990; Bardoux et al., 2003b).

Our study has some limitations related to the experimental model. Experiments were performed in the *db/db* mouse, one of the most widely used rodent models of type 2 diabetes. It allows for the study of the early stage of diabetic nephropathy but like other genetic or dietary models of type 2 diabetes, it does not reproduce features of evolved human diabetic kidney disease (Betz et al., 2014). Also, we chose to limit the experiment to 12 weeks in order to avoid hydronephrosis or dehydration related to high urine output caused by diabetes and V2R treatment. Accordingly, we cannot establish whether vasopressin and the V2R are still involved in progression of diabetic nephropathy at more advanced stages of the disease.

5. Conclusion

We showed that in an animal model of type 2 diabetes, treatment with a selective vasopressin V2R antagonist improves albuminuria and partially prevents hyperfiltration or kidney hypertrophy, which are all features of incipient diabetic nephropathy. In addition to supporting a role of V2R activation in the early phase of diabetic kidney disease, these results provide a pathophysiological basis for the association observed in epidemiological studies of type 2 diabetes subjects between high vasopressin/copeptin levels and albuminuria or kidney function decline. The study also suggests putative new therapeutic strategies for renal protection in patients, based on pharmacological blockade of V2R or inhibition of endogenous vasopressin secretion by high water intake.

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Conflicts of interest

The authors declare that there is no conflict of interest associated with this manuscript. Dr. Roussel has been a consultant or is on the speakers' bureau for AstraZeneca, Janssen, Eli Lilly, Sanofi, Merck Sharp and Dohme, and Novo-Nordisk, and has received research funding from Sanofi and Amgen.

Contribution statement

REB, CT, CC, GV, RR, FAG and NB contributed to the acquisition and analysis of data and helped to review the article. REB, GV, RR, LB and NB contributed to the design and conception of the study, provided data interpretation and wrote the article. All authors approved the final version of the manuscript.

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Table 1: Physiological parameters at baseline and at the end of the study

	db/m	*non-treated db/db*	*Treated db/db*

Body weight (g)			
baseline	21.4 ± 0.8	32.3 ± 3.7*	33.5 ± 1.5*
week 12	26.4 ± 2.3	31.0 ± 5.2	31.8 ± 5.0
Fasting blood glucose (mg/dl)			
baseline	128 ± 11	239 ± 135*	311 ± 89*
week 12	131 ± 10	510 ± 72*	497 ± 68*
Glycosuria (mmol/day)			
baseline	-	3.19 ± 1.42	1.82 ± 0.58
week 12	-	5.05 ± 2.32	4.87 ± 2.08
Osmolar excretion (mosm/day)			
baseline	1.8 ± 0.4	6.9 ± 2.2*	5.4 ± 2.4*
week 12	2.7 ± 0.5	8.8 ± 3.6*	8.4 ± 2.1*
Systolic blood pressure (mm Hg)	119 ± 13	113 ± 18	118 ± 17

Data are presented as mean ± SD (n=7 per group). Statistics are ANOVA followed by Fisher's *post hoc* test: *significantly different ($p<0.05$) from *db/m*. Blood pressure data were obtained at week 3 of treatment.

Table 2: Effect of V2R antagonist on heart and kidney weight

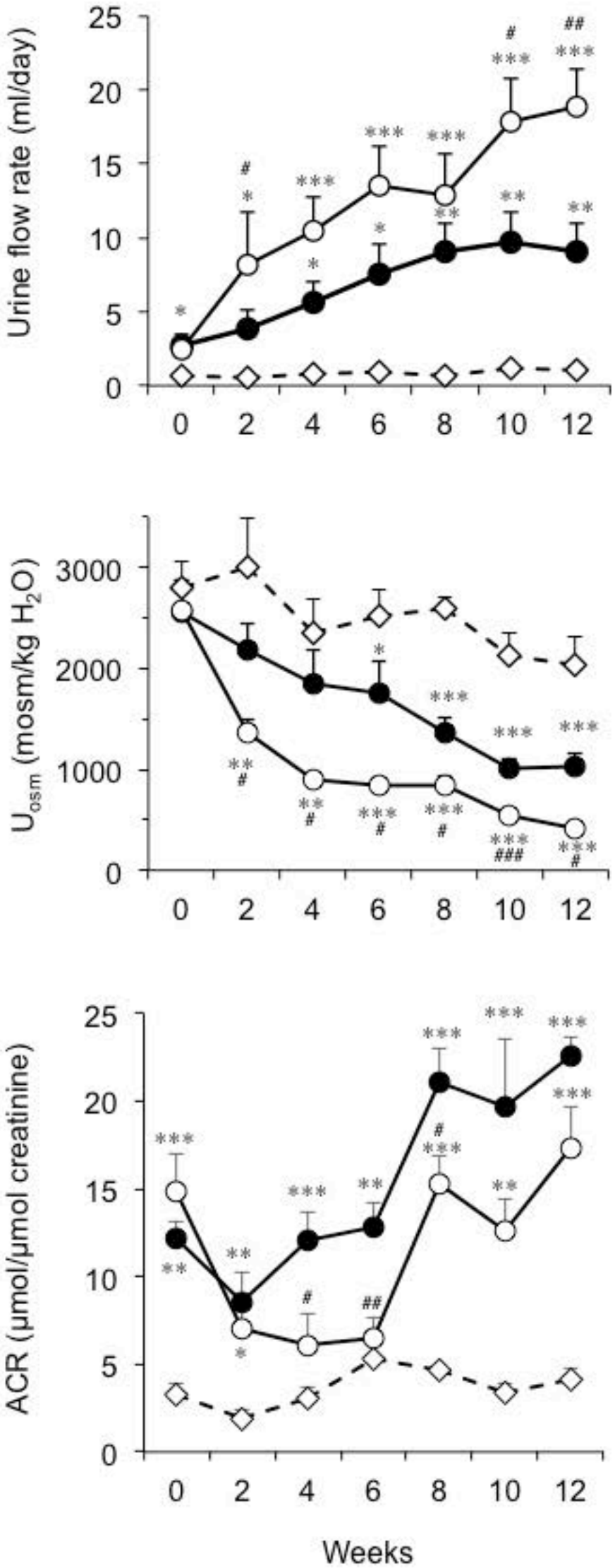
	db/m	*non-treated db/db*	*Treated db/db*
Kidney			
absolute weight (mg)	260 ± 22	267 ± 19	238 ± 15$^{*\#}$
relative weight (mg/10g bw)	94 ± 8	83 ± 10	76 ± 11*
Heart			
absolute weight (mg)	126 ± 10	133 ± 57	121 ± 25
relative weight (mg/10g bw)	45 ± 3	43 ± 23	41 ± 9

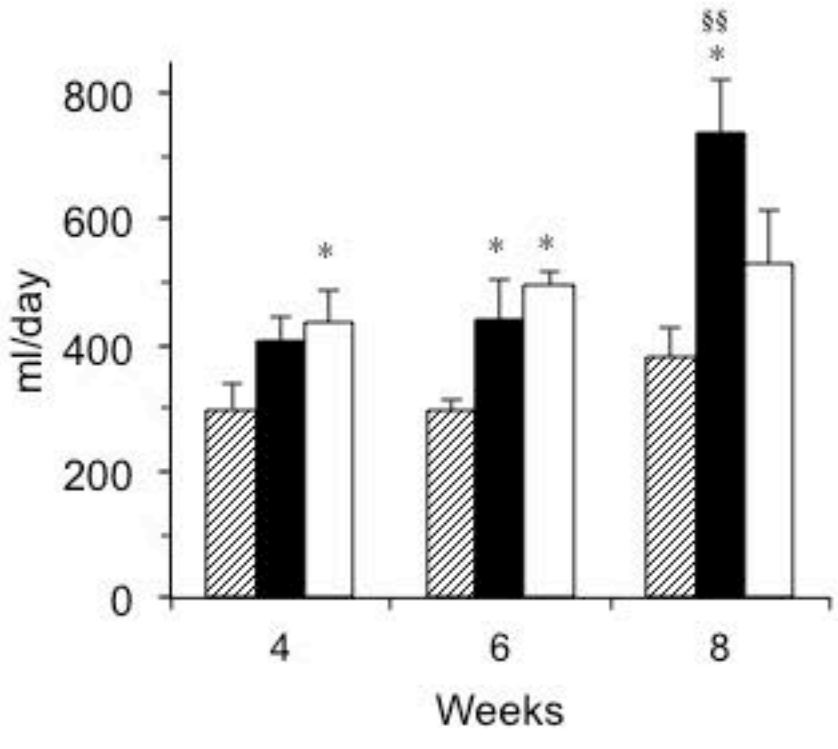
Data are presented as mean ± SD, (n=7 per group). Statistics are ANOVA followed by Fisher's *post hoc* test: *significantly different ($p<0.05$) from *db/m* and Student's *t*-test between non-treated *db/db* and treated *db/db* mice: $^{\#}$significantly different ($p<0.05$) from non-treated *db/db*

Figure legends

Figure 1. Evolution of urine flow rate, urine osmolality (U_{osm}), and albuminuria expressed as albumin to creatinine ratio (ACR) in non-diabetic mice (diamonds), non-treated diabetic *db/db* mice (closed circles) and diabetic *db/db* mice treated with the V2R antagonist, 30 mg/kg/day then 45 mg/kg/day from week 9 to 12 (open circles). Data expressed as mean ± SEM. Statistics are ANOVA for repeated measures followed by Fisher *post hoc* test: *versus non-diabetic *db/m* mice and $^{\#}$versus non-treated diabetic *db/db* mice.

Figure 2. Creatinine clearance at 4, 6 and 8 weeks of treatment with the V2R antagonist in non-diabetic mice (dashed bars), non-treated diabetic mice (black bars) and diabetic mice treated with the V2R antagonist, 30 mg/kg/day (white bars). Data expressed as mean ± SEM. Statistics are ANOVA for repeated measures and Fisher *post hoc* test: *significantly different ($p<0.05$) from *db/m* on the same week, § significantly different ($p<0.05$) from previous weeks in the same group.


Urine flow rate (ml/day)
25
20
15
10
5
0
0
2
4
6
8
10
12
U_{osm} (mosm/kg H_2O)
3000
2000
1000
0
ACR (µmol/µmol creatinine)
Weeks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800
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