Journal of Diabetes and Its Complications xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Diabetes and Its Complications

journal homepage: WWW.JDCJOURNAL.COM

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

Ray El Boustany [a,b,c], Christopher Taveau [a,b,d], Catherine Chollet [a,b,d], Gilberto Velho [a], Lise Bankir [a,b,d], François Alhenc-Gelas [a,b,d], Ronan Roussel [a,b,e,f], Nadine Bouby [a,b,d,*]

[a] INSERM, UMRS_1138, Centre de Recherche des Cordeliers, Paris, France
[b] Université Pierre & Marie Curie, Paris, France
[c] Danone Research-R&D Waters, Hydration and Health Dept., Palaiseau, France
[d] Université Paris Descartes, Paris, France
[e] Université Paris Diderot, Paris, France
[f] Département de Diabétologie-Endocrinologie-Nutrition, DHU FIRE, Hôpital Bichat, AP-HP, Paris, France

ARTICLE INFO

Article history:
Received 12 January 2017
Received in revised form 7 February 2017
Accepted 3 April 2017
Available online xxxx

Keywords:
Vasopressin
V2 receptor
Antagonist
Diabetic nephropathy
Type 2 diabetes

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 glycemia 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.

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.[1] An expanding set of data suggests that the vasopressin–hydration axis plays a role in onset and progression of chronic kidney disease (CKD).[2] 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.[3–5] High circulating copeptin concentration was also associated with the development and progression of diabetic nephropathy in patients with type 2 diabetes,[6–10] and with prevalence of ESRD in subjects with long-standing type 1 diabetes.[11,12] Experimental data suggest 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).[13,14] 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.[13] 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.[15] In a rat model of type 1 diabetes, vasopressin was shown to contribute to microalbuminuria through its V2R activation.[16] 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

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.

* Corresponding author at: UMRS_1138, Centre de Recherche des Cordeliers, 15 rue de l'Ecole de Médecine, Paris, France.
E-mail address: nadine.bouby@crc.jussieu.fr (N. Bouby).

http://dx.doi.org/10.1016/j.jdiacomp.2017.04.005
1056-8727/

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-h light/dark cycle and had free access to water.

Seven-week old mice of both strains underwent left nephrectomy under pentobarbital anesthesia (Nembutal®, 0.6 mg/10 g BW), in order to increase the vulnerability of the remnant kidney.[17] Two weeks after the surgical procedure, animals were housed in metabolic cages (Techniplast, Lyon, France) for two consecutive 24-h 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-h 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-h 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 12th 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, $p < 0.05$) (Fig. 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 ($p = 0.02$) (Fig. 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 (Fig. 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) (Fig. 2).

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. Blood pressure data were obtained at week 3 of treatment.
* Significantly different ($p < 0.05$) from *db*/*m*.

Please cite this article as: El Boustany R, et al. Antagonism of vasopressin V2 receptor improves albuminuria at the early stage of diabetic nephropathy in a mouse model of type 2 d.... *Journal of Diabetes and Its Complications* (2017), http://dx.doi.org/10.1016/j.jdiacomp.2017.04.005