Carbamazepine affects water and electrolyte homoeostasis in rat—similarities and differences to vasopressin antagonism

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Abstract

Background. Carbamazepine (CBZ) is a drug widely used in the therapy of epilepsy and mood disorders. One frequently observed side effect is hyponatraemia. The role of vasopressin in hyponatraemic action of CBZ is discussed controversially. In this study, we tested the influence of CBZ on water and salt homoeostasis in rat under different hydration states and under vasopressin 2 receptor (V2R) antagonism by satavaptan to elucidate the renal and vasopressin independent action of CBZ.

Methods. CBZ-treated rats were investigated on metabolic cages after (i) 6 day with ad libitum fluid intake, (ii) moderate water load and (iii) water restriction. The effect of satavaptan was tested in clearance experiments under continuous saline infusion in anaesthetized rats after CBZ pretreatment.

Results. Compared to controls, CBZ induced a higher urinary flow rate which was most pronounced (20-fold) after water load and significantly elevated (2-fold) after 10-h water restriction. In addition, CBZ consistently increased renal sodium loss but failed to decrease plasma sodium concentration. In the presence of satavaptan, urinary flow and natriuresis were further increased by CBZ, while there was no differential effect on urea excretion and anion gap.

Conclusions. At the investigated dose (50 mg/kg body weight), CBZ did not induce hyponatraemia or antidiuresis in the rat. However, depending on the hydration state, it induced an increased water and electrolyte loss. Its enhanced influence on urinary flow and natriuresis in the presence of satavaptan suggests additional renal targets for CBZ, independent of vasopressin signalling.

Keywords: carbamazepine; epithelial transport; ion homoeostasis; kidney; vasopressin

Introduction

Carbamazepine (CBZ) is a drug that has been prescribed since the 1960s [1]. In neurology, it is used in the treatment of trigeminal neuralgia [2] as well as epilepsy and bipolar disorders. In the central nervous system (CNS), CBZ reduces neuronal hyperexcitability and elicits its action mainly by inhibition of neuronal Na⁺ and, to a lesser extent, also other channels and transmitter systems [3, 4]. One side effect of CBZ therapy, especially in elderly patients with co-medication, is hyponatraemia [5]. This electrolyte disturbance is rather frequent, even though in most cases asymptomatic. As it may cause severe problems, it occasionally requires therapy withdrawal. Interestingly, in the past, CBZ has been successfully applied in the treatment of central diabetes insipidus [6] to substitute the insufficient action of vasopressin (antidiuretic hormone, ADH) in these patients. Therefore, hyponatraemia in CBZ therapy has been discussed as syndrome of inadequate antidiuretic hormone release indicating an increased release of ADH and a consecutive hyponatraemia by dilution [7]. Since patients and healthy volunteers did not show consistently elevated but also decreased vasopressin plasma concentrations under CBZ treatment [8–13], alternatively direct effects of CBZ on renal tubular electrolyte transport are discussed [14, 15]. Although rats are commonly used in studies investigating the antiepileptic properties of CBZ, little is known about the metabolic response of these animals to CBZ. In the comparative discussion of metabolic data, sex differences in renal function have to be considered [16]. Parallel to our study in female rats, de Bragança et al. [17] investigated male rats for the effects of CBZ. They found that CBZ increased osmotic water permeability in isolated rat inner medullary collecting duct and alleviated lithium-induced nephrogenic diabetes insipidus. A direct effect on the vasopressin 2 receptor (V2R)-protein G complex was proposed [17].

In this study, we investigated the effects of CBZ on water and electrolyte homoeostasis under different hydration states. We worked with the V2R antagonist satavaptan to test whether there is V2R-independent CBZ action in the kidney.

Materials and methods

Animals

Female Wistar rats (200–300 g) were bred and maintained in our animal research facilities, fed ad libitum and housed under a 12-h light/dark cycle. All procedures were approved by the institutional animal research and care committee.

Metabolic protocols

Animals were trained for gavage with 2% tylose (vehicle) and on metabolic cages 3 days before the start of the metabolic experiments. Three
different experimental protocols were carried out on metabolic cages: (i) 6 day application of CBZ, (ii) 4 day CBZ application together with a bolus water load on Day 1 and Day 4 and (iii) CBZ application under 10-h water restriction. During experiments, either 2% tylose [1 μL/g body weight (BW)] or 50 mg/kg BW CBZ (50 mg/mL CBZ in 2% tylose, 1 μL/g BW) was applied by gavage (peroral). The suspensions of CBZ were ground in a zirconium dioxide mill. This dose resulted in plasma concentrations of 13.9 ± 1.3 μg/mL, (n = 9), measured 5 h after application. Time line and settings of protocols are described in detail in the Results section. On metabolic cages, food and water intake were monitored, and urine and faeces were collected and analysed. At the end of the respective experimental protocol, animals were sacrificed under inhalative anæsthesia (2–3% isoflurane) by final bleeding and plasma samples were analysed.

Clearance experiment
Vehicle or CBZ was administered by gavage 2 h prior to the experimental period. The experiment itself was then performed in anaesthetized rats (pentobarbital sodium, 80 mg/kg BW, intraperitoneal (i.p.); maintenance under 2% isoflurane). Experiments were carried out between 8 am and 1 pm. Anaesthetized rats were placed on a heating plate to maintain the body temperature at 37°C. Blood pressure was measured via an arterial catheter (femoral artery). Through a venous catheter (femoral vein), rats received an infusion of 0.9% NaCl solution at a rate of 12 μL/g BW/h for 30 min (control period). The V2-antagonist satavaptan (25 μg/mL in 0.9% NaCl) was administered at a dose of 0.3 mg/kg BW/h for another 120 min (12 μL/g BW/h). The infusion was controlled by a perfusion pump. Body temperature, breathing rate and heart rate as well as systolic blood pressure were measured every 15 min throughout the experiment. Urine was collected in vials via a bladder catheter in 15-min interval. At the end of the experiment, a final blood sample was taken from the abdominal vena cava. For determination of acid–base homeostasis, blood was taken from the tail vein.

Urine and plasma analysis
Plasma and urinary creatinine concentrations were measured enzymatically (Creatinine PAP; Biocon Diagnostics) either in a spectrophotometer or a Roche Modular P analyser (Roche Diagnostics). In addition, urine creatinine concentrations were measured by the method of Jaffe. Plasma and urinary concentrations of sodium and potassium were measured by flame photometry. Urine and plasma osmolality were determined using a micro-osmometer (Fiske). For the clearance experiment, urea and chloride concentrations were measured on the Roche Modular P analyser. Blood gas analysis was performed on an Ecosys II blood gas analyser (Eischweiler). ADH was measured by radioimmunoassay in a service unit (Medizinisches Labor, Bremen). The assay detection range has been 1.9–52 pg/mL. Reference values for the plasma osmolality range of our samples (296–300 mOsm/kg) are given as 4.0–12.0 pg/mL. Samples were collected as ethylenediaminetetraacetic acid plasma 5 h after the administration of CBZ or vehicle. A 2 mL blood sample was collected within <10 s from the abdominal aorta after consecutive anæsthesia by 3.5% isoflurane inhalation and 80 mg/kg BW i.p. injection of pentobarbital sodium.

Statistical analyses
All data are given as mean ± SEM. Unpaired t-test was used to test for differences between vehicle- and CBZ-treated animal groups. In the clearance experiment, paired t-test was used to test for differences induced by application of satavaptan compared to pre-control values. One-way analysis of variance was used to test differences in parameters between days in the water load experiment. A P-value ≤0.05 was accepted for statistical significance. n indicates the number of independent experiments.

Results

6 Day CBZ treatment
Animals were treated as illustrated in Figure 1A. Samples from the last day were analysed and data are shown in Figure 1 and Table 1. Under these experimental conditions, rats showed no hyponatraemia. There was no evidence of inadequate vasopressin action (hyperhydration). In contrast, plasma osmolality was slightly increased indicating rather the opposite effect on water balance. The analysis of kidney parameters did not show any changes in urinary flow and urine osmolality over 24 h. Creatinine clearance, as a measure of glomerular function, was also unaltered. With respect to tubular handling of Na⁺ and K⁺, we determined the ratio of UNa/Ucrea and UK/Ucrea. Since plasma values for Na⁺ and creatinine were not different between groups, this ratio is a measure of fractional excretion, i.e. inverse to the tubular reabsorption of the respective solute. Compared to vehicle values, CBZ treatment increased UNa/Ucrea by 27% and UK/Ucrea by 29%, respectively. Also, the increased osmotic clearance and a more negative free water clearance support a distinct effect of CBZ on water and salt balance in rats. This prompted us to continue our investigations by challenging water balance by either water load or water restriction.

Moderate water load
Animals were treated as illustrated in Figure 2A. Parameters are shown in Figure 2 and Table 2. Analysis of urine volume revealed a difference in urinary flow rate at Day 1.
in the CBZ-treated animal group. This diuresis was accompanied by a dilution of urine Na and creatinine concentrations and therefore did not interfere with absolute Na⁺ excretion at the time resolution of a 24-h sampling period. There were no differences in UNa/Ucrea. Also at the end of Day 4, no changes in plasma electrolytes were visible. The second additional water load at Day 4 did not elicit significant changes and even the discrete changes in osmole handling seemed to be normalized in CBZ-treated rats.

Taking circadian changes in water and salt homoeostasis into account, we analysed Day 1 of this experiment in more detail: Figure 3 shows the experimental protocol and the data for different sampling periods. Data from the vehicle group on Days 2 and 3 (without water load) served as a reference for circadian behaviour of the investigated parameters. CBZ or vehicle application as well as water load fell into the light phase, the inactivity phase in rodents, visible as low urinary output during daylight hours compared to the night. Water load in the control group more than doubled these values and additional CBZ led to a massive 20-fold increase in urine production. In parallel, urine osmolality dropped in the first 5 h after water load in the CBZ group compared to the vehicle group and remained decreased.

Renal sodium handling showed pronounced circadian differences with lower Na⁺ excretion in the inactivity phase. The water-loaded vehicle-treated group did not alter Na⁺ handling. The water-loaded and CBZ-treated group doubled the tubular Na⁺ loss in the first 5 h after water load, whereas in the following 16 h, including the active night phase, compensation already took place with reduced sodium excretion. Renal potassium handling showed a similar but less pronounced circadian pattern. In the case of potassium, water load induced an increased K⁺ loss in both the vehicle- and the CBZ-treated group, but only in the CBZ-treated group was this followed by compensatory potassium saving in the active phase.

**CBZ treatment under water restriction**

In a next series of experiments, we investigated the effect of CBZ under water restriction. Figure 4 and Table 3 illustrate the protocol and respective data. While control rats continuously decreased urine output, the urinary flow after CBZ treatment remained 1.6-fold higher. Correspondingly, urine osmolality was slightly reduced after CBZ treatment. CBZ clearly affected saluresis under these conditions. Renal Na⁺ loss was almost doubled and K⁺ loss was 1.5-fold increased. The plasma values again displayed no hyponatraemia but a slightly higher plasma osmolality. There was no change in creatinine clearance but a higher osmotic clearance and a more negative free water clearance.

### Table 1. Plasma (P) values, clearance (C) and excretion parameters after 6 day application of CBZ in conscious rat, 24-h urine sampling period

<table>
<thead>
<tr>
<th>6 Day (end point)</th>
<th>Vehicle</th>
<th>CBZ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNa (mmol/L)</td>
<td>145 ± 0.8</td>
<td>145 ± 0.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>PK (mmol/L)</td>
<td>3.5 ± 0.1</td>
<td>3.8 ± 0.4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Psm (mOsm/kg H2O)</td>
<td>298 ± 2</td>
<td>304 ± 2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Crea (μL/min/g BW)</td>
<td>6.8 ± 1.4</td>
<td>7.2 ± 0.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cosm (μL/min/g BW)</td>
<td>0.13 ± 0.02</td>
<td>0.17 ± 0.01</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>CH2O (μL/min/g BW)</td>
<td>−0.09 ± 0.02</td>
<td>−0.12 ± 0.00</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Urine flow rate</td>
<td>0.046 ± 0.004</td>
<td>0.052 ± 0.005</td>
<td>n.s.</td>
</tr>
<tr>
<td>Osmole excretion</td>
<td>40 ± 5.1</td>
<td>52 ± 2.3</td>
<td>&lt;0.05</td>
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</tbody>
</table>

n = 7

*a.s., not significantly different.*
**V2R antagonism and CBZ treatment**

Figure 5A illustrates the experimental protocol for the combined V2R antagonism and CBZ treatment experiments. Saline infusion began simultaneously with application of anaesthesia. Urine sampling started at Time 0 in 15-min intervals and continued throughout the experiment. The graphs present data which were obtained at these intervals within a time period spanning 15 min prior to satavaptan application and continuing through 60 min post application. The first plotted data points (15–30 min sampling) reflect the baseline values (Figure 5B–E). Table 4 gives plasma values at the end of the experiment. Clearance values, urine flow rate and osmole excretion were calculated from data obtained at the sampling interval from 75 to 90 min.

Compared to vehicle treatment, the urinary flow rate was already increased in the CBZ group under baseline conditions before application of satavaptan. V2 antagonism led to a steep increase in both groups, with a delayed onset of some 20 min. In parallel, urine osmolality dropped in both groups and reached similar low values. Urine Na+/creatinine ratio tended to be higher in CBZ-treated animals. Satavaptan significantly increased Na+ excretion at 90 min. With respect to K+, there was no significant difference between the two groups. Whereas UK/Ucrea stayed stable in the vehicle group, it decreased in the CBZ group. The urine Na+/K+ ratio indicated a clear

**Table 2** Plasma (P) values, clearance (C) and excretion parameters after 4 day application of CBZ and water load at Day 1 and Day 4 in conscious rat, 21-h urine sampling period<sup>a</sup>

<table>
<thead>
<tr>
<th>Water load (end point Day 4)</th>
<th>Vehicle</th>
<th>CBZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>P&lt;sub&gt;Na&lt;/sub&gt; (mmol/L)</td>
<td>141 ± 0.5</td>
<td>141 ± 0.8</td>
</tr>
<tr>
<td>P&lt;sub&gt;K&lt;/sub&gt; (mmol/L)</td>
<td>3.3 ± 0.6</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>P&lt;sub&gt;osm&lt;/sub&gt; (mOsm/kg H&lt;sub&gt;2&lt;/sub&gt;O)</td>
<td>309 ± 1</td>
<td>308 ± 2</td>
</tr>
<tr>
<td>C&lt;sub&gt;crea&lt;/sub&gt; (μL/min/g BW)</td>
<td>7.0 ± 0.9</td>
<td>6.2 ± 1.0</td>
</tr>
<tr>
<td>C&lt;sub&gt;osm&lt;/sub&gt; (μL/min/g BW)</td>
<td>0.13 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>CH&lt;sub&gt;2&lt;/sub&gt;O (μL/min/g BW)</td>
<td>0.041 ± 0.011</td>
<td>0.051 ± 0.008</td>
</tr>
<tr>
<td>Osmole excretion (nOsm/min/g BW)</td>
<td>39 ± 3.8</td>
<td>36 ± 6.4</td>
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</table>

<sup>a</sup>n.s., not significantly different.

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**Fig. 3.** Renal function at higher temporal resolution after acute CBZ treatment and water load (cf. Figure 2). (A) Sampling and treatment protocol. Black bar: reference group without water load taken from Days 2–3. Gavage of either 2% tylose (white bars) or 50 μg/g BW CBZ (in 2% tylose, grey bars) at 9 am and additional water load (12 μL/gBW) at noon and sampling on metabolic cages at noon, 2pm, 5pm and 9am. (B) Water load led to a slight increase in urinary flow in the vehicle group in the first 2 h but to a massive increase in the CBZ-treated group and a corresponding decrease in urine osmolality. (C) CBZ increased Na<sup>+</sup> excretion (Ex<sub>Na</sub>) in the first 5 h after application and a consecutive Na<sup>+</sup> conservation was observed in the following 16 h. (U<sub>Na</sub>/U<sub>crea</sub>: urinary Na<sup>+</sup> concentration normalized by urinary creatinine concentration as a measure of fractional excretion). (D) Water load increased urinary K<sup>+</sup> excretion (Ex<sub>K</sub>) followed in the CBZ-treated group by potassium saving in the night phase. (UK/U<sub>crea</sub>: urinary K<sup>+</sup> concentration normalized by urinary creatinine concentration as a measure of fractional excretion.) Data shown as mean ± SEM, n = 5. *, Significant effect of CBZ treatment. #, Significant effect of water load versus water ad libitum.
difference between CBZ- and vehicle-treated rats. Independent of V2R antagonism, CBZ increased this value throughout the experimental period and did not interfere with the satavaptan-induced increase in Na+/K+ ratio at the 75 and 90 min time points. In addition, urea handling was also strongly influenced by CBZ in the presence of satavaptan. While V2R antagonism in the control group increased Uurea/Ucrea as expected, renal urea handling remained unaffected by satavaptan in the CBZ pretreated rats. We also analysed the relationship between the main anion Cl− and the cations K+ and Na+ to get indirect information on the contribution of NH4+, HCO3− and H2PO4−. Astonishingly, this ratio was already higher in the CBZ-treated group before satavaptan treatment and increased only weakly under V2R antagonism. In contrast, in the control group, Na+ and K+ exceeded Cl− by >2-fold prior to application of satavaptan. The ratio offset increased even further in the presence of satavaptan, reaching similar values to the CBZ group at 90 min. Blood gas analysis at the end of the experiment depicted in a Davenport diagram (Figure 5E). It revealed slightly more alkalotic values in the CBZ-treated group with a shift in the HCO3− concentration to values above the 25 mmol/L HCO3− buffer line.

Further analysis of the plasma revealed decreased sodium and increased potassium concentrations reflecting K+ sparing natriuresis. The sustained urea excretion in CBZ-treated animals prior to the addition and in the presence of satavaptan application was reflected by decreased plasma urea concentrations (Table 4).

Taken together, this experiment shows dependent and independent action of CBZ and satavaptan.

**ADH measurements**

To test whether the effect of CBZ was related to ADH suppression, we performed a separate experiment. We
determined the effect of CBZ on plasma concentration of ADH. Five hour after the administration of vehicle, the concentration of ADH was $14 \pm 5$ pg/mL ($n = 5$). CBZ-treated animals showed no significant difference with a concentration of $20 \pm 5$ pg/mL ($n = 5$).

**Discussion**

Carbamazepine is still an indispensable drug in antiepileptic therapy. Its side effects on Na$^+$ and water metabolism are well described. However, it is still not clearly
defined how CBZ interferes with renal function, salt transport and volume regulation. To avoid the unwanted abort of CBZ therapy in patients due to derailment of electrolyte balance, it is necessary to understand the underlying mechanisms and to develop preventive strategies in an appropriate animal model. In this in vivo study, we investigated whether the rat could be an appropriate model and how CBZ interferes with renal function within the therapeutical concentration range. We show the effects of CBZ and their relation to vasopressin antagonism on tubular transport of salt and water. We found that CBZ acts on renal function in addition to vasopressin signalling but also mimics vasopressin antagonism. CBZ therapy in the rat did not necessarily induce hyponatraemia and hypervolaemia. Therefore, at first sight, the rat appears not to be a representative model to mimic these side effects of CBZ in human therapy. However, the results clearly show interference of CBZ with renal function which are beyond interference with vasopressin control and will prompt us to further investigate the respective mechanisms on a cellular level.

The controversy about the mode of action of CBZ on salt and water transport most likely reflects effects of the drug at multiple distinct targets. Unfortunately, there is a lack of functional data but still, a number of appealing hypotheses on these putative targets have been suggested. On the one hand, these targets might be expressed in the hypothalamus leading, namely increased diuresis, Na+ and K+ excretion. The key findings could also indicate that the rats were in a compensatory state after an acute initial disturbance of salt or water balance.

Therefore, we looked in closer detail at the sequence of events in the initial phase of treatment and under defined water intake. In this second approach, we provided a water load to the animals. Interestingly, CBZ potentiated the effect of water load on diuresis in the first 5 h, while control animals were able to compensate their water load easily by reduced intake during the remaining daily sampling period. Hence, CBZ seemed to interfere with the acute control of diuresis acting like a vasopressin antagonist. The effect was most prominent in the first 2 h.

The excretions of Na+ and K+ were already counter regulated in the following period of the day. It is important to note that diuresis and Na+ excretion follow the diurnal cycle of animal activity and renal function [22]. Urinary flow as well as electrolyte excretion are both lower during daytime, the inactive phase of rodents, thus acute effects of CBZ could be underestimated or overlooked if judged by longer periods of sampling and analysis.

Also under the third condition (water restriction), CBZ had consistent acute effects on renal salt and water handling, namely increased diuresis, Na+ and K+ excretion.

In fact, a diuretic response to CBZ has previously been observed but not further interpreted [17]. In this study, the effect of CBZ on lithium-induced diabetes insipidus was investigated in male rats in vivo and on inner medullary collecting duct function in vitro. The key finding in vivo was that CBZ attenuated the diuretic effect of lithium treatment and that the ‘antidiuretic’ action of CBZ in the presence of lithium was mediated via V2R signalling and AQP2 expression. On first sight, these findings are contradictory to our data, however, a closer look at the respective results [17] reveal that CBZ treatment induced an increase in urine flow rate by >2-fold if compared to controls and that the effect of CBZ on AQP2 expression was only detected in the presence of lithium [17].

Our measurements of plasma ADH concentrations showed no significant differences between vehicle-treated and CBZ-treated animals at 5 h after administration, the phase of clearly visible effects on salt and water handling. In contrast to a suspected decrease of ADH under CBZ, we observed even a trend to higher values. This speaks in favour of a renal CBZ action, independent from central effects on salt and water intake as also suggested from

<table>
<thead>
<tr>
<th>Satavaptan (Time 75–90 min)</th>
<th>Vehicle</th>
<th>CBZ</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNa (mmol/L)</td>
<td>146 ± 0.8</td>
<td>141 ± 1.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PK (mmol/L)</td>
<td>3.8 ± 0.0</td>
<td>4.3 ± 0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>PCI (mmol/L)</td>
<td>113.3 ± 2.5</td>
<td>118.3 ± 2.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Pura (mmol/L)</td>
<td>6.2 ± 0.5</td>
<td>4.7 ± 0.3</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Posm (mOsm/kg H2O)</td>
<td>316 ± 3</td>
<td>324 ± 4</td>
<td>n.s.</td>
</tr>
<tr>
<td>Crea (µL/min/g BW)</td>
<td>8.9 ± 0.5</td>
<td>9.5 ± 0.5</td>
<td>n.s.</td>
</tr>
<tr>
<td>Cosm (µL/min/g BW)</td>
<td>276 ± 32</td>
<td>328 ± 61</td>
<td>n.s.</td>
</tr>
<tr>
<td>CHZO (µL/min/g BW)</td>
<td>−276 ± 32</td>
<td>−327 ± 61</td>
<td>n.s.</td>
</tr>
<tr>
<td>Urine flow rate</td>
<td>0.679 ± 0.121</td>
<td>1.050 ± 0.086</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Osmole excretion (µL/min/g BW)</td>
<td>88 ± 9.7</td>
<td>106 ± 18.8</td>
<td>n.s.</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

* Plasma samples were taken at the end of the experiment. Urine samples were collected between 75 and 90 min. n.s., not significantly different.
human data [10]. It is important to note that normal values of ADH strongly depend on plasma osmolality and may be significantly biased by the method of anaesthesia and blood sampling which both might cause an acute change in blood pressure and a respective increase in ADH within minutes [23]. Since we did not collect the samples in trained conscious animals via an implanted catheter, we cannot exclude a systematic overestimation of ADH concentrations in both groups or even blunting of a CBZ effect.

In conclusion, our data from three metabolic experiments suggested that the rat experienced a disturbance of renal function that was most prominent with the acute onset of CBZ treatment and compensated to a large extent within 1 day of treatment cycle as well as within a long-term treatment period. If these findings are transferred to the normal human patient, susceptibility for the renal action of CBZ could be dependent on the hydration state and be prominently visible in the first hours after drug ingestion and particularly at the onset of therapy.

The renal action of CBZ could occur at several levels. One major regulatory pathway is the ADH receptor and its downstream signalling via cyclic adenosine monophosphate (cAMP) and the respective effector proteins like AQP2 and membrane ion transport proteins serving Na⁺ transport in the thick ascending limb (TAL) and collecting duct [24]. If CBZ would exclusively interfere with this pathway, we would expect that its action would be similar to V2R inhibition. In the presence of alternative targets along the nephron, CBZ should be able to act additively or to modify the effects of V2R inhibitors on tubular transport. This was tested in the fourth experimental protocol. In this experiment, we first measured the acute effect of CBZ under strictly controlled intake (saline infusion) under anaesthesia. Independent of appetite and thirst (behaviour), CBZ caused diuresis and a change in ion handling \([\text{UCl}]/(\text{UNa} + \text{UK})\). Subsequently, both vehicle and CBZ groups received additional and continuous infusion of the V2R antagonist satavaptan. With continuous infusion and accumulation of satavaptan, we observed its typical effects on renal salt and water handling. In the vehicle group, this resulted in an increase in urinary flow as well as in urinary Na⁺, K⁺ and urea excretion and corroborated findings reported in male rats [25]. The animals with the additional presence of CBZ showed three different phenotypes of combined CBZ and satavaptan action. Some parameters converged towards the same values as in the vehicle group, indicating common targets and pathways. Some parameters were influenced in an additive manner, indicating additional points of action. And, even more surprisingly, some parameters which consistently reacted to satavaptan in the vehicle group only hardly reacted to satavaptan in the presence of CBZ. This led us to the conclusion that CBZ action in the kidney is pleiotropic and involves V2R-dependent and -independent pathways and target proteins.

Under control conditions, CBZ initially increased urine flow and tended to increase Na⁺ and K⁺ excretion, suggesting ADH suppression or V2R antagonism as one mechanism. Addition of the V2R antagonist satavaptan revealed that CBZ led to a higher increase of urine flow, urine Na⁺ excretion and urine Na⁺/K⁺ ratio despite the full inhibition of V2R. Therefore, CBZ affected renal function on top of V2R antagonism. The effect of satavaptan on urea excretion and on the ratio of \(\text{UCl}/(\text{UNa} + \text{UK})\) was abolished if animals were pretreated with CBZ. The second messenger pathways of V2R signalling, however, are principally the same for water and urea transport [26]. Hence, these data offer two pathways of CBZ action. One interfering with renal function in line with vasopressin action, and another affecting renal targets downstream or independent of vasopressin agonism. In any case, it is unlikely that the effects of CBZ can be pinned down to a single target or mechanism.

Our measurements of the ratio of \(\text{UCl}/(\text{UNa} + \text{UK})\) further suggested that CBZ might also interfere with acid–base metabolism. In the diagnosis of electrolyte disturbances, this ratio is mostly used in the interpretation of acid–base disturbances [27]. On the one hand, if the kidney excretes more NH₄⁺ (with Cl⁻ as counter ion), this ratio increases. On the other hand, if HCO₃⁻ excretion rises (with Na⁺ and K⁺ as counter ion), this ratio decreases. In our hands, satavaptan treatment increased the ratio in the vehicle- as well as in the CBZ-treated group, whereas the CBZ group already started with clearly elevated values. In addition, the CBZ-treated group showed slightly elevated plasma bicarbonate and lower urea concentrations compared to the respective vehicle group indicating a misbalanced synthesis and handling of HCO₃⁻ and NH₄⁺. Vasopressin action on ammonium and bicarbonate metabolism is ample and not restricted to the kidney [28]. Since it has been shown that vasopressin reduces HCO₃⁻ absorption in the TAL [29], inhibition by satavaptan would increase HCO₃⁻ absorption and thereby accordingly increase the ratio \(\text{UCl}/(\text{UNa} + \text{UK})\). There are a variety of targets which have to be analysed for their sensitivity towards CBZ. Based on our observations, a potential effect of CBZ on renal cAMP handling is one candidate. It has been published that CBZ was able to decrease cAMP in brain tissues [30]. This would be compatible with the compromised handling of water and urea in the collecting duct and of Na⁺ distal to the loop of Henle. From its nature as an ion channel blocker, CBZ would also be a candidate to interfere with renal K⁺ channel function thereby inhibiting Na⁺ and NH₄⁺ absorption along the nephron. Finally, the transporters for urea and HCO₃⁻ are candidates worth examining although the urinary balance of anions and cations showed a clear dependence on V2R antagonism.

Taken together, this study shows for the first time the complex acute effects of CBZ \textit{in vivo} on the rat renal salt and water handling. It comprises both V2R-dependent and -independent ways of action and points out that the acute analysis of urine volume, Na⁺ excretion and the ratio of urine Cl⁻/(Na⁺ + K⁺) could be indicators in patients for their susceptibility towards CBZ-induced changes in electrolyte balance.

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