Effects of the adenosine A1 receptor inhibitor FK 838 on proximal tubular fluid output in rats

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Abstract

Background. Adenosine A1 receptor blockade has been suggested as a treatment in conditions with sodium and fluid retention because it increases urinary Na+ excretion and increases proximal tubular fluid output. In the present study, we examine the time course for the renal responses to adenosine A1 receptor blockade in order to investigate whether the effects may be prolonged and not just temporary.

Methods. The acute effects of the adenosine A1 receptor inhibitor FK 838 on segmental tubular Na+ handling were examined by a renal clearance technique in conscious chronically instrumented rats. Lithium clearance (CLi) was used as a clearance marker of proximal tubular fluid output.

Results. Acute adenosine A1 receptor inhibition did not affect the glomerular filtration rate (GFR) significantly. In contrast, the inhibition led to significant increases in CLi (from 290±28 to 431±28 ml/min/100 g), fractional Li+ excretion (FE Li) (from 33±2 to 47±3%) and fractional Na+ excretion (FENa) (from 0.44±0.07 to 2.03±0.42%). Sodium excretion, expressed as a fraction of proximal tubular fluid output (CNa/CLi), rose from 1.3±0.2 to 4.2±0.4%, suggesting that the natriuretic effect was supported by inhibition of distal nephron Na+ reabsorption. All values returned to baseline values during the clearance study and thereby indicated that none of these effects of adenosine A1 blockade were long lasting.

Keywords: adenosine A1; FK 838; tubulo-glomerular feedback

Introduction

In the kidney, there are at least two different subtypes of adenosine receptors, known as A1 and A2. Selective stimulation of A1 receptors leads to vasoconstriction of the afferent arteriole in the nephron, whereas stimulation of A2 receptors leads to vasodilation [1]. Adenosine causes net vasoconstriction since A1 receptor-mediated vasoconstriction prevails over the A2 receptor-mediated vasodilation [2]. Several studies have shown that infusion of adenosine causes sodium and water retention [3,4], whereas infusion of specific adenosine A1 blockers has been shown to stimulate sodium excretion [5–10].

The natriuretic effect of adenosine A1 blockade can be ascribed to several intra-renal effects of adenosine. Adenosine A1 blockade has a direct inhibitory effect on proximal tubular Na+ reabsorption, resulting in increased proximal tubular fluid output [5–8]. In addition, it is believed that adenosine A1 receptor stimulation play an essential role in the so-called tubulo-glomerular feedback (TGF) mechanism, i.e. the afferent arteriolar vasoconstriction that is seen in response to an increase in NaCl delivery to macula densa [11]. Adenosine A1 receptor blockade should therefore oppose any decrease in glomerular filtration rate (GFR) that would normally occur in response to an increased proximal tubular fluid output, leaving GFR unaltered or slightly elevated [5,6,9,12–14].

Due to its natriuretic effect, combined with increased proximal tubular fluid output, adenosine A1 blockade has been suggested as a treatment in conditions with
sodium and fluid retention such as congestive heart failure [15,16]. It has also been suggested that adenosine A<sub>1</sub> blockade may support the natriuretic effect of diuretics that normally has only a short-lasting effect due to Na<sup>+</sup> loss that leads to decreases in GFR and proximal tubular fluid output [15,16]. However, the chronic effects of adenosine A<sub>1</sub> blockade on segmental tubular Na<sup>+</sup> handling and Na<sup>+</sup> homeostasis is insufficiently clarified. In particular, it is unknown whether adenosine A<sub>1</sub> blockade may lead to a chronic increase in the proximal tubular fluid output.

The aim of the present study was to study the effect of adenosine A<sub>1</sub> blockade in conscious rats in order to avoid the confounding effect of anaesthesia and examine whether the increase in proximal tubular fluid output in response to adenosine A<sub>1</sub> receptor blockade is temporary or long lasting.

**Subjects and methods**

**Animals**

Specific pathogen-free female Wistar rats (M&B, Eiby, Denmark) weighing between 250 and 280 g were used. Animals were housed in a room with 12:12 h artificial light cycle, lights on 8 a.m. to 8 p.m., temperature 21 ± 2°C and humidity 55 ± 2%. The rats were fed a wet mash diet containing 200 mmol of Na<sup>+</sup> and 200 mmol of K<sup>+</sup> per kg dry weight. The diet was fed for 2–3 weeks prior to the experiment and, 3 days before the experiment, the diet was changed to a diet containing lithium chloride (10–12 mmol/kg dry weight). The diet was fed for 2–3 weeks prior to the experiment and, 3 days before the experiment, the diet was changed to a diet containing lithium chloride (10–12 mmol/kg dry weight) in order to obtain measurable plasma lithium concentrations without influencing renal function [17]. Rats had free access to food and tap water throughout the studies.

One to two weeks before the experiment, the animals were anaesthetized with intraperitoneal neuroleptic anaesthesia, Hypnorm® (Janssen Pharmaceuticals, Belgium) (fentanyl citrate 0.315 mg/ml + fluanxone 10 mg/ml) 400 μl/kg + Dormicum® (Hoffman-La Roche, Switzerland) (midazolam 5 mg/ml) 800 μl/kg. Using semi-aseptic surgical techniques, sterile Tygon<sup>®</sup> catheters (Norton Performance Plastics, Arkon, OH) were advanced into the abdominal aorta and the inferior vena cava via the femoral vessels. A sterile chronic suprapubic catheter was implanted into the bladder. All catheters were produced and fixed, with minor modifications, as described previously [18,19]. The arterial and venous catheters were sealed with 50% glucose solution containing heparin (100 U/ml). The rats were transferred to a restraining cage and connected to infusion pumps via the venous catheter and to a blood pressure transducer via the arterial catheter. Through the venous catheter, a 25 mM glucose solution containing heparin (100 U/ml) at a rate of 1 ml/min was given to keep the arterial catheter open. Through the venous catheter, a 25 mM glucose solution was infused at a rate of 39 ml/min before the treatment periods and at 29 μl/min after start of treatment infusion (10 ml/min) in order to maintain a urine flow necessary for accurate bladder emptying. [3H]Inulin (2.5 μCi/ml, Amersham, Rainham, UK) and LiCl (12 mmol/l) were infused at a rate of 10 μl/min as markers of the GFR and proximal tubular fluid output, respectively. The clearance markers were dissolved in 150 mM NaCl. Altogether, the rats received an intravascular fluid infusion of 50 μl/min throughout the experiment.

The infusion was initiated by an equilibration interval of 120 min, during which the different clearance markers were given. This was followed by a baseline urine collection of 40 min. FK 838 infusion was then started and urine was collected in four successive experimental periods of 40 min. The vehicle group was treated similarly except that FK 838 was replaced with vehicle alone. Blood samples (200 μl) were drawn from the arterial catheter after 120, 160, 240 and 320 min. A 2 ml sample of blood was drawn at 320 min for angiotensin II (ANG II) determination. The red blood cells from the blood samples were resuspended in saline and reinjured after each blood sample. Mean arterial blood pressure was recorded continuously using a pressure transducer (Baxter Uniflow, Bendley Laboratories, Europe BV, Udem, The Netherlands) connected to a pre-amplifier and PC registration. Approximately 20% of the rats were excluded due to occlusion of one or more of the various catheters.

**Analysis**

Urine volume was determined by gravimetry. Li<sup>+</sup> concentration was determined in plasma and urine by flame emission photometry and atomic absorption spectrophotometry, respectively. [3H]Inulin in plasma and urine was determined by liquid scintillation counting (1900 CA, Packard Instruments, Meriden, USA). Sample (15 μl) and 285 μl of water were mixed with 2.5 ml of scintillation liquid (Ultima Gold, Packard Instruments, Meriden, USA). Calculation of d.p.m. was performed by automatic efficiency control.
To determine ANG II immunoreactivity in plasma, a specific antibody (Ab5-030682) produced by P. Christensen and kindly provided by P. Bie, Department of Physiology and Pharmacology, University of Southern Denmark, was used in a final concentration of 1:100 000 as described by Grove et al. [20] with minor modifications for use in rats. Briefly, Isolute C8 cartridges (Microlab, Denmark) were activated with 4% CH₃COOH in 99.9% ethanol, followed by methanol, and finally water. A 100 μl aliquot of plasma or plasma containing ANG II tracer (for recovery estimation) was acidified with 800 μl of 4% CH₃COOH and added to the cartridges. The cartridges were washed with 2 ml of water followed by desorption of the adsorbed angiotensin with 0.2 ml of 4% CH₃COOH in 60% ethanol and again by 0.2 ml of the same solution. The fluid was evaporated and the extract redissolved in 500 μl of assay buffer. Plasma samples were incubated with antibody for 24 h and with tracer ¹²⁵I-labelled ANG II (Department of Clinical Physiology, Glostrup Hospital, Denmark) for another 24 h. Separation of antibody-bound from free radioiodinated ANG II was carried out by means of charcoal adsorption. The antibody used did not cross-react with des-Asp¹-angII (ANG III), and <0.1% with ANG I. Mean recovery of ANG II was 75%. The intra- and inter-assay coefficients of variation were 11 and 18%, respectively.

**Calculations**

Renal clearances (C) and fractional excretions (FE) were calculated by the standard formula:

\[
C = \frac{U \times V}{P} \quad \text{and} \quad \text{FE} = \frac{C}{GFR}
\]

where U is the urine concentration, V is the urine flow rate, P is the plasma concentration and GFR is the glomerular filtration rate.

The renal clearance of lithium (CLi) was used as an index of proximal tubular fluid output [21]. The CLi technique is based on the findings that under normal circumstances, the fractional delivery of lithium from the proximal tubule in excess of the fractional delivery of water is largely matched by lithium reabsorption in the loop of Henle, while lithium reabsorption beyond the loop is quantitatively insignificant. The CLi technique, like other methods, has its advantages and its shortcomings [21]. In particular, small changes in CLi cannot be interpreted as necessarily resulting from changes in proximal reabsorption, and agents known to affect loop of Henle transport should be avoided. In rats, conditions which lower sodium or potassium excretion should also be avoided or at least interpreted with extreme caution. Despite these caveats, in the vast majority of situations, CLi is a useful semi-quantitative, directional marker of changes in delivery of sodium and water from the proximal tubules [21]. Since our study did not involve any of the circumstances listed above, we consider the lithium clearance technique to be a reliable method in the present investigation, although it cannot be excluded that part of the increase in FELi and CLi in response to FK 838 could be due to diminished distal Li⁺ reabsorption.

**Data presentation and statistics**

All values are presented as mean±SEM. Overall statistical analyses were performed by one-way ANOVA (between groups) or one-way ANOVA for repeated measurements (within groups). Subsequently, the effect of FK 838 was evaluated by comparison of the baseline period with the first experimental period using Student’s paired t-test. Differences were considered statistically significant at the 0.05 level.

**Results**

In the baseline period, mean arterial pressure (MAP) was similar in the two groups, i.e. 118±4 and 116±3 mmHg in the FK 838 and the time control group, respectively. It decreased within the first 60 min of FK 838 infusion by ~6–8 mmHg (P<0.05) and remained at this level until the end of the experiment. In the time control group, MAP remained unaltered. There were no changes in plasma sodium, plasma potassium or haematocrit during administration of FK 838 (data not shown).

GFR showed a tendency to increase (from 869±53 to 932±56 μl/min/100 g body weight) from baseline to the first period after start of FK 838, but the increase was statistically insignificant (Figure 1). GFR remained constant in the time control group. CLi (used as an index of proximal tubular fluid output [21]) rose from 290±28 to 431±28 μl/min/100 g body weight (P<0.001) and FE Li rose from 0.33±0.02 to 0.47±0.03 in the first period after start of FK 838 infusion (Figure 1), which clearly demonstrated an inhibitory effect of FK 838 on proximal tubular Na⁺ reabsorption. The effect on CLi and FE Li decreased gradually during the experiment and was no longer present in the last period in which CLi was 336±25 μl/min/100 g body weight and FE Li was 36±2%. Sodium clearance (CNa) rose from 4.0±0.9 to 17.4±3.2 μl/min/100 g body weight (P<0.001) in the first period after start of FK 838 infusion and then decreased slowly towards the baseline value at the end of the experiment. FENa was increased by a factor of 4–5 in the first period after FK 838 administration, but the effect gradually faded and was hardly visible at the end of the experiment (Figure 1). Sodium excretion, expressed as a fraction of the distal delivery (CNa/CLi), showed an almost similar pattern, which suggests that the effect on urinary Na⁺ excretion was due to a combined effect of increased Na⁺ delivery from the proximal tubule (CNa) and inhibition of distal nephron Na⁺ reabsorption. FEK showed a temporary rise similar to that observed for sodium. FEv also showed a temporary rise that was due primarily to an increase in proximal tubular fluid output since the fractional distal water excretion (VEC/Li) was unaltered. Plasma ANG II increased from 220±35 to 439±62 pg/ml (P<0.01) during treatment with FK 838. The increase in this Na⁺-retaining hormone is in agreement with the return of FE Li, CLi and FENa towards baseline.

**Discussion**

The main finding of the present study was an increase in CLi and FELi and an increase in CNa/CLi and FENa.
Fig. 1. Glomerular filtration rate (GFR), fractional excretions of lithium (FE\textsubscript{Li}), sodium (FE\textsubscript{Na}), potassium (FE\textsubscript{K}) and water (FE\textsubscript{V}), and excretions of sodium (C\textsubscript{Na}/C\textsubscript{Li}) and water (V/C\textsubscript{Li}) expressed as a fraction of the distal delivery during one control period (0–40 min) and during infusion of vehicle or FK 838 (40–200 min). Control period vs peak effect (first experimental period) (paired Student's t-test): **P < 0.01; ***P < 0.001.
without any significant changes in GFR. This shows that the natriuretic effect of FK 838 was due to a combined effect on the proximal tubules and the distal nephron. During the 160 min in which FK 838 was infused, C\textsubscript{Li}, F\textsubscript{Ei} and C\textsubscript{Na}/C\textsubscript{Li} returned to their baseline levels. This shows that the effect of FK 838 on proximal and distal tubular fluid output was temporary within the time limits of the study.

Our results are in agreement with previous studies that have shown an increase in proximal tubular fluid output during adenosine A\(_1\) receptor blockade in rats [5,6,9,12–14]. It is believed that adenosine A\(_1\) receptor stimulation is responsible for the so-called TGF response, i.e. the afferent arteriolar vasoconstriction that is seen in response to an increase in NaCl delivery to macula densa [22]. Adenosine A\(_1\) receptor blockade should therefore lead to an increase in GFR or it should at least prevent a decrease in GFR in response to an increase in proximal tubular fluid output [15,23]. This was also seen in our study where GFR showed a slight, although statistically insignificant, increase in spite of a pronounced increase in C\textsubscript{Li}. A more pronounced increase in GFR in response to FK838 was probably counteracted by the decrease in blood pressure and the increase in plasma ANG II. Our results are therefore not against the hypothesis that adenosine mediates TGF.

Our results are in agreement with the notion that adenosine A\(_1\) blockers exert a direct inhibitory effect on proximal Na\(^+\) reabsorption [5–9,12,13]. In the first period after FK 838 administration, F\textsubscript{Ei} rose from 0.33 to 0.47, which clearly shows that FK 838 inhibited proximal tubular Na\(^+\) reabsorption.

Several studies have shown that C\textsubscript{Na}/C\textsubscript{Li} is increased during adenosine A\(_1\) receptor blockade [5,6,13,24]. Only one study found no change in distal nephron Na\(^+\) reabsorption [12]. However, because C\textsubscript{Li} is increased during adenosine A\(_1\) blockade, it is uncertain whether the increase in C\textsubscript{Na}/C\textsubscript{Li} is due to saturation of Na\(^+\) reabsorption in the distal nephron or due to direct inhibition of Na\(^+\) reabsorption in the distal nephron. Therefore, it cannot be finally settled whether adenosine A\(_1\) blockade has a direct inhibitory effect on the distal nephron.

According to the discussion above, there seems to be general consensus that the diuretic and natriuretic effects of adenosine A\(_1\) receptor blockade can be ascribed to its effects on the proximal tubular Na\(^+\) reabsorption and on the TGF mechanism. The combination of these two effects leads to an unopposed increase in proximal tubular fluid output that is unique for adenosine receptor blockers and makes these potentially interesting for clinical use. Adenosine A\(_1\) blockade has been suggested as a treatment in conditions with fluid retention due to congestive heart failure, and it has been suggested that adenosine A\(_1\) blockade may support the natriuretic effect of diuretics that normally have a short-lasting effect due to Na\(^+\) loss leading to a decreases in GFR and proximal tubular fluid output [15,16]. However, while most studies focus on the acute effects of adenosine A\(_1\) receptor blockade, a possible clinical use of adenosine A\(_1\) blockade may rather call for attention to its prolonged effects on kidney function and Na\(^+\) homeostasis. The present results clearly showed that prolonged administration of FK 838 led to activation of antinatriuretic mechanisms such as a decrease in blood pressure and increase in plasma ANG II, and Na\(^+\) excretion returned to baseline within the time limits of the clearance study. This is in agreement with the notion that the Na\(^+\) excretion cannot stay elevated for a prolonged time. An increase of the Na\(^+\) excretion above the normal level would lead to Na\(^+\) loss that eventually would lead to a lowering of the Na\(^+\) excretion. It has been shown that knockout mice may survive without adenosine A\(_1\) receptors [22,23]. Clearly this shows that their Na\(^+\) excretion matches their Na\(^+\) intake. In one study in adenosine A\(_1\) receptor knockout mice, the urinary Na\(^+\) excretion was monitored in metabolic cages for 24 h [22]. The results showed that there was no difference between wild-type mice and knockout mice. The finding in another study that the Na\(^+\) excretion of the knockout mice was higher than that of the wild-type mice may reflect a lowered response to anaesthesia and operation in knockout mice rather than a chronically higher Na\(^+\) excretion in these animals [23]. Van Buren et al. [6] examined the effect of a single dose and repeated administration for 7 days of an adenosine A\(_1\) receptor blocker in hypertensive patients. Clearance measurements were carried out on days 1 and 7. On both days, Na\(^+\) excretion rose shortly after administration of the drug but it returned to baseline after 2 h. This is a further indication that although the acute natriuretic effect can be repeated for many days, it is short lasting.

In theory, the proximal tubular fluid output may stay elevated for a prolonged time provided that the increased proximal Na\(^+\) output is reabsorbed in the distal nephron [25]. However, in the present study, there was no indication that C\textsubscript{Li} and F\textsubscript{Ei} would stay elevated, and there was no indication that distal nephron Na\(^+\) reabsorption was elevated. On the contrary, it was inhibited. Nevertheless, it has been reported that C\textsubscript{Li} was chronically increased in lithium-treated patients given theophylline, which is an unspecific adenosine A\(_1\) and A\(_2\) receptor blocker [26]. The reason for this observation remains obscure. In the study by van Buren et al. in which hypertensive patients were treated with an adenosine A\(_1\) receptor blocker for 7 days, F\textsubscript{Ei} rose acutely after administration of the adenosine A\(_1\) blocker, but the baseline level after 7 days of treatment was the same as when the treatment started. This supports the notion that adenosine A\(_1\) blockade does not lead to a long-lasting increase in proximal tubular fluid output. However, in clinical conditions with lowered proximal tubular fluid output and fluid retention due to cardiovascular diseases, the blocker may very well act differently. This remains to be investigated.

It is concluded that in conscious unstressed rats, acute adenosine A\(_1\) receptor inhibition by FK 838 led to a significant natriuresis that was caused by inhibition of
proximal tubular Na⁺ reabsorption, possibly with a contribution from inhibition of distal nephron Na⁺ reabsorption. The increased proximal tubular fluid output and the increased urinary Na⁺ excretion returned to baseline values during the clearance study, indicating that none of these effects was long lasting.

The present results clearly showed that prolonged administration of FK 838 led to activation of antinatriuretic mechanisms such as a decrease in blood pressure and increase in plasma ANG II, and Na⁺ excretion returned to baseline within the time limits of the clearance study.

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Conflict of interest statement. None declared.

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