Natriuretic effect of caffeine: assessment of segmental sodium reabsorption in humans

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ABSTRACT

In order to assess the intrarenal mechanisms responsible for the natriuretic action of caffeine, the renal clearances of $^{51}$Cr-EDTA (used as a measure of glomerular filtration rate (GFR)) and lithium (used as an index of end-proximal fluid delivery) were measured in eight healthy males before (control period) and immediately after (experimental period) a 400 mg oral dose of caffeine (given over 90 min) or placebo. In caffeine-treated subjects, the fractional excretion of sodium rose from $1.00 ± 0.25%$ in the control period to $1.47 ± 0.18%$ in the experimental period, while corresponding values on the placebo day were $1.04 ± 0.16%$ and $0.70 ± 0.07%$ respectively. GFR was unchanged following either caffeine or placebo. When compared with the placebo day, caffeine caused increases in lithium clearance (experimental period values: caffeine, $37 ± 1$ ml/min; placebo, $28 ± 2$ ml/min; $P < 0.001$), the fractional excretion of lithium (caffeine, $34 ± 1%$; placebo, $26 ± 2%$; $P < 0.001$) and the sodium/lithium clearance ratio (used as an index of the fraction of sodium delivered to the distal nephron that escapes reabsorption therein: caffeine, $4.4 ± 0.3%$; placebo, $2.8 ± 0.2%$; $P < 0.001$). These results suggest that reduced fractional sodium reabsorption in both the proximal tubule and the distal nephron contributes to the acute natriuretic effect of caffeine. The data also confirm the importance of controlling caffeine intake when investigating renal function using lithium clearance.

INTRODUCTION

It is now generally accepted that caffeine increases sodium excretion (e.g. [1,2]). However, despite its widespread use as a dietary constituent and drug adjuvant, the intrarenal mechanisms responsible for the natriuretic effect of caffeine remain to be determined, and conflicting views exist over the respective roles of altered renal haemodynamics and tubular reabsorption [1,3]. A suggestion that the proximal tubule might be involved came from an early study by Thomsen and Schou [4] in which it was reported that caffeine led to a rise in lithium clearance ($C_{Li}$), a variable now known to provide an index of end-proximal fluid delivery [5,6]. On the basis of that single report, caffeine-containing drinks have been prohibited in the vast majority of subsequent physiological and clinical studies involving measurement of $C_{Li}$. It is worth noting, however, that Thomsen and Schou [4] gave no details of the caffeine dosage, the magnitude of the change in $C_{Li}$ or the number of subjects studied. Moreover, a subsequent short report could find no effect of caffeine on $C_{Li}$ in healthy volunteers [7].

In view of these conflicting findings, and of the potential importance of the need to control caffeine intake in $C_{Li}$ studies, the present study was undertaken to determine the effect of a moderate dose of caffeine on the glomerular filtration rate (GFR) and tubular sodium reabsorption in healthy subjects. Sodium reabsorption in

Key words: caffeine, lithium clearance, natriuresis, proximal tubule.
Abbreviations: $C_x$, clearance of x; FE$_x$, fractional excretion of x; GFR, glomerular filtration rate.
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METHODS

Subjects and protocol

Experiments were performed on eight normotensive non-smoking subjects (males; age range 21–52 years) previously screened by medical examination and routine haematological and biochemical analyses. The study was approved by the local Ethical Committee (Riverside) and subjects gave informed, written consent. Each subject was studied on two occasions, separated by at least 1 week. For the 4 days leading up to each study day, subjects remained on their normal diet, but were asked to refrain from eating foods with particularly high or low sodium content (a list being given for guidance). To assess dietary sodium, urine was collected for the 24 h preceding each study day. During the 24-h urine collection, as well as on the study day itself, subjects abstained from alcohol and from all caffeine-containing foodstuffs (list provided). At 22.00 hours on the pre-study day, a single dose of 300 mg of lithium carbonate (Delandale Laboratories, Canterbury, Kent, U.K.) was taken. The next morning, after a light breakfast, subjects reported to the laboratory at 9.00 hours. An intravenous saline at 2 ml/kg body weight; thereafter, urine flow rate and thereby eliminate bladder emptying errors. Subjects were supine except when micturating or drinking.

After 2 h, by which time the urine flow rate had stabilized, a 1 h (control) clearance period was initiated. At the end of the control period, a dose of 100 mg of caffeine (PP Products, Welwyn Garden City, Herts., U.K.) was taken, and this was repeated at 30-min intervals for 90 min (total caffeine intake of 400 mg). Clearance measurements were then repeated during the first 1 h after the discontinuation of caffeine (experimental period). Venous blood samples were taken at hourly intervals throughout.

Exactly the same procedures were performed on the second study day, except that placebo tablets were taken. The order in which the two study days were performed was varied from subject to subject.

Analyses

Sodium and potassium concentrations were measured by flame photometry (model 543; Instrumentation Laboratory, Warrington, U.K.); lithium concentrations by atomic absorption spectrophotometry (model 151; Instrumentation Laboratory); and $^{51}$Cr-EDTA activities by $\gamma$ spectroscopy (Autogamma 5550 series; Canberra Packbourne, Pangbourne, Berks., U.K.).

Calculations and statistics

Clearances ($C_r$) were calculated using the standard formula $C_r = U_x \cdot V/P_x$, where $U_x$ is the urine concentration of x, $V$ is the urine flow rate and $P_x$ is the plasma concentration of x. The renal clearance of $^{51}$Cr-EDTA was taken to be a measure of GFR. Fractional excretions (FE$_x$) were calculated as $C_r$/GFR.

Values are presented as means $\pm$ S.E.M. Comparisons between the caffeine and placebo days, with respect to changes in a given variable with time (control period compared with experimental period), were made using two-way ANOVA with repeated measures. Post-hoc assessment was performed using the Student–Newman–Keuls test. Statistical significance was taken at the 5% level.

RESULTS

24-h urine collections

The sodium content of the 24-h urine sample collected immediately before the caffeine study day was 146 $\pm$ 15 mmol, and that before the placebo day was 152 $\pm$ 9 mmol. Respective 24-h creatinine excretions were 15.8 $\pm$ 1.2 mmol and 14.8 $\pm$ 1.0 mmol. On this basis, sodium intake was adjudged to be similar in the periods leading up to the two study days.

Plasma electrolytes

Table 1 shows the plasma concentrations of sodium, potassium and lithium, at the start of the control period and at the end of the experimental period, on the two study days.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Control period</th>
<th>Experimental period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na$^+$ (mmol/l)</td>
<td>137 $\pm$ 1</td>
<td>135 $\pm$ 1</td>
</tr>
<tr>
<td>K$^+$ (mmol/l)</td>
<td>4.4 $\pm$ 0.3</td>
<td>3.9 $\pm$ 0.2</td>
</tr>
<tr>
<td>Li$^+$ (mEq/l)</td>
<td>117 $\pm$ 11</td>
<td>96 $\pm$ 7</td>
</tr>
</tbody>
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Values (means $\pm$ S.E.M.; n = 8 subjects) are shown for the start of the control period and the end of the experimental period. *P < 0.05 for significant interaction between treatment and time compared with placebo day.
study days. The fall in plasma lithium concentration with time was significantly greater ($P < 0.05$) on the day on which caffeine was ingested.

**Clearance data**

Sodium excretion rates are shown in Figure 1. Baseline values were similar on the two days, but during the period 30–60 min after the start of caffeine ingestion sodium excretion was already significantly elevated; it remained elevated throughout the study period. Subsequent data are presented only for the control and experimental periods.

During the experimental period, $\text{FE}_{\text{Na}}$ was significantly higher on the caffeine day than on the placebo day, and there was a significant interaction between treatment and time (Figure 2), indicating that the natriuresis resulted, at least partly, from a reduction in fractional tubular reabsorption. Fractional water excretion also increased after caffeine administration. However, fractional potassium excretion was not significantly altered.

GFR, calculated from the renal clearance of $^{51}$Cr-EDTA, was stable on both days and was unaffected by caffeine (Figure 3). During the experimental period, $C_{\text{Li}}$ (an index of end-proximal fluid delivery) was significantly higher on the caffeine day than on the placebo day, and there was a significant interaction between treatment and time. These comments also applied to $\text{FE}_{\text{Li}}$.

Finally, $C_{\text{Na}}/C_{\text{Li}}$, used as an index of the fraction of sodium delivered to the distal nephron that escapes reabsorption therein, was also significantly elevated after caffeine, and again there was a significant interaction between treatment and time.

**DISCUSSION**

The present study confirmed that a moderately high dose of caffeine, ingested over a 90-min period, caused a substantial acute increase in sodium excretion and an accompanying diuresis. Caffeine is a major dietary constituent, and its natriuretic and diuretic effects have been recognized for some time, yet only fragmentary evidence is available concerning its renal site(s) of action. The present study has attempted to clarify this issue by assessing the effect of caffeine on the renal clearance of $^{51}$Cr-EDTA, a more accurate measure of GFR than that usually employed (creatinine clearance), and by using the $C_{\text{Li}}$ technique to assess segmental sodium reabsorption. The use of $C_{\text{Li}}$ as a measure of the volume of glomerular
Figure 3 Effects of caffeine on (a) $^{51}$Cr-EDTA clearance (GFR), (b) $C_{Li}$, (c) $FE_{Li}$ and (d) the $C_{Na}/C_{Li}$ ratio
Measurements were made during the 1 h control period (C; open circles) and the 1 h experimental period (E) following oral caffeine (closed circles) or placebo (shaded circles) administration. Values are means ± S.E.M. ($n = 8$); *$P < 0.001$ for caffeine compared with placebo. NS, not significant.

filtrate reaching the end of the proximal tubule is based on the premise that lithium is reabsorbed in the proximal tubule in the same proportion as water, but that none is reabsorbed in the loop of Henle or beyond [8]. Although micropuncture studies have shown that lithium reabsorption in the proximal tubule actually lags slightly behind that of water, and that a small proportion of filtered lithium is reabsorbed in the loop of Henle [9–11], these small errors largely cancel out and the consensus is that, under normal conditions, $C_{Li}$ does fulfil its purpose as a reasonable estimate of end-proximal fluid delivery [5,6]. An exception to this is under conditions of sodium restriction, when lithium can be reabsorbed additionally in the collecting duct, at least in rats [12]; however, none of the subjects in the present study was on a low sodium intake, as indicated by the 24-h excretion rates.

Our finding of a substantial increase in sodium excretion without any measurable change in GFR indicates that the caffeine-induced natriuresis resulted largely from inhibition of fractional tubular reabsorption, as confirmed by the marked increase in $FE_{Na}$. This finding, together with the previous demonstration that caffeine is without effect on renal plasma flow in humans [1], challenges the widely held view that renal haemodynamic effects are involved in the natriuresis [3,13] (although we cannot discount the possibility that higher doses of caffeine might increase GFR).

Both $C_{Li}$ and $FE_{Li}$ increased substantially after caffeine. This indicates that at least part of the overall reduction in sodium reabsorption occurred in the proximal tubules, leading to an increase in end-proximal fluid delivery. One factor underlying the reduction in proximal tubular reabsorption might be the well documented pressor effect of acute caffeine administration [1,3]. Increases in renal perfusion pressure, by increasing renal interstitial hydrostatic pressure, can inhibit proximal tubular reabsorption, at least in deep nephrons [14]. However, the dose of caffeine used in the present study results in only a small change in mean arterial pressure [1], and this mechanism is unlikely to be the sole cause of the increase in $FE_{Li}$ [15]. Caffeine is known to be a non-specific antagonist of adenosine receptors [3], and it is reasonable to propose that $A_2$ receptor antagonism contributes to its proximal effect: in vivo clearance and micropuncture studies in rats have indicated that specific $A_2$-adenosine receptor antagonists can inhibit proximal
tubular reabsorption [16–18], through mechanisms as yet unknown. A further effect of A<sub>1</sub>-adenosine receptor antagonism is inhibition of the tubulo-glomerular feedback mechanism [18–20]. This action could explain how an increase in the volume of fluid arriving at the distal tubule was sustained in the present study without eliciting a tubulo-glomerular feedback-mediated reduction in GFR.

As well as reduced proximal tubular reabsorption, our results also point to an effect of caffeine on the distal nephron. Although absolute sodium reabsorption in the distal nephron increased substantially (otherwise the raised distal delivery would have resulted in a vastly greater increase in sodium excretion than that observed), the marked increase in the C<sub>GFR</sub>/C<sub>Li</sub> ratio (an index of the fraction of sodium delivered to the distal nephron that was excreted in the urine) indicates that the fractional distal reabsorption of sodium fell following caffeine administration. The increase in absolute distal sodium reabsorption would be anticipated, partly as an inherent response of the distal nephron to the increased load and partly as a consequence of A<sub>1</sub>-adenosine receptor antagonism: in vitro evidence suggests that A<sub>1</sub> receptor stimulation by adenosine can inhibit reabsorption in the thick ascending limb of the loop of Henle [21] and in the cortical and medullary collecting ducts [22,23]. Indeed, long-term treatment with specific A<sub>1</sub>-adenosine receptor antagonists is associated with increased fractional sodium reabsorption in the distal nephron to the extent that, despite sustained inhibition of proximal tubular reabsorption, overall sodium excretion returns to normal (see [24]). The present finding that, in the acute situation, fractional distal sodium reabsorption was reduced suggests that, in the case of caffeine, additional factors (possibly related to its A<sub>2</sub>-adenosine receptor antagonism) must have been operating to offset the above effects. Caffeine has no inhibitory effect on either plasma renin activity [1,2,25] or aldosterone secretion [2,26], which appears to rule out a role for suppressed activity of renin–angiotensin–aldosterone system. Nor is there any evidence that caffeine stimulates atrial natriuretic peptide secretion [2]. On the basis of current information, therefore, the observed acute reduction in distal fractional reabsorption must remain unexplained. Although it has been suggested that caffeine stimulates renal prostaglandin synthesis [3], which could in theory inhibit sodium reabsorption in the distal nephron, direct evidence is lacking.

Finally, the practical implications of the present findings for the use of the C<sub>Li</sub> technique in physiological and clinical investigations should be considered. In 1984, Thomsen [27] recommended that coffee, tea, cola, cocoa and other caffeine-containing beverages be avoided in all studies of C<sub>Li</sub>, on the basis of anecdotal evidence that caffeine increases C<sub>Li</sub>. Since then, in hundreds of C<sub>Li</sub> studies in patients and healthy subjects, this stricture has been followed. Our findings indicate that the recommendation was sound: despite the inconvenience caused, avoidance of caffeine (or, alternatively, standardization of caffeine intake) is necessary if this factor is not to bring unwanted variability to C<sub>Li</sub> values. Our findings also provide a basis for earlier claims [28,29] that variations in caffeine intake can influence the plasma lithium concentration in patients receiving prophylactic lithium treatment and thereby either reduce the effectiveness of treatment or precipitate a lithium overdose.

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