Original Paper

Stimulation of Intestinal Cl⁻ Secretion Through CFTR by Caffeine Intake in Salt-Sensitive Hypertensive Rats

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Key Words

Caffeine • Salt-sensitive hypertension • Intestinal sodium absorption • CFTR • CAMP

Abstract

Background/Aims: High salt consumption is a major risk factor for hypertension, and sodium homeostasis is regulated by both intestinal sodium absorption and urinary sodium excretion. Chronic caffeine intake has been reported to attenuate salt-sensitive hypertension by promoting urinary sodium excretion; however, its exact role in intestinal sodium absorption remains unknown. Here, we investigated whether and how chronic caffeine consumption antagonizes salt-sensitive hypertension by inhibiting intestinal sodium absorption. **Methods:** Dahl salt-sensitive rats were fed 8% NaCl chow and 0.1% caffeine in their drinking water for 15 days. The blood pressure and fecal sodium content were measured. The effect of caffeine on the movement of Cl⁻ in enterocyte cells was determined with the Ussing chamber assay. **Results:** Rats that were treated with caffeine displayed significantly lower mean blood pressure and higher fecal sodium content than the controls. Consistent with these findings, caffeine intake decreased fluid absorption by the intestine in the fluid perfusion experiment. Further, the results from the Ussing chamber assay indicated that caffeine promoted Cl⁻ secretion through enterocyte apical cystic fibrosis transmembrane conductance regulator (CFTR), and thus inhibited sodium absorption. Moreover, depletion of cAMP or inhibition of CFTR completely abolished the effect of caffeine on Cl⁻ secretion. **Conclusion:** The results indicate that chronic caffeine consumption reduces sodium absorption by promoting CFTR-mediated Cl⁻ secretion in the intestine, which contributes to the anti-hypertensive effect of caffeine in salt-sensitive rats.

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Introduction

Hypertension is the main cause of a series of cardiovascular diseases, including stroke, heart failure, and coronary heart disease. It is predicted that hypertension will become the leading cause of death worldwide by 2030 [1]. High salt intake is a major risk factor for the onset and development of hypertension, as salt intake directly corresponds to blood pressure (BP) [2]. In particular, it has been estimated that 30–50% of hypertensive individuals and 25% of normotensive individuals are sensitive to salt [3]. Until 2010, the global mean sodium intake was 3.95 g/day, which is nearly twice the upper limit of 2 g/day that is recommended by the World Health Organization (WHO); moreover, the intake seems to be on the increase [4]. The strategies for lowering salt intake include the dissemination of health education programs among the public and administration of diuretic and natriuretic drugs; however, it seems that it is still hard to meet the WHO requirements [5]. Thus, a more effective, convenient and accessible approach to decreasing sodium intake is urgently needed to prevent the onset and development of salt-sensitive hypertension.

Sodium homeostasis is affected by both sodium absorption and excretion [6]. Although renal sodium excretion has been studied for years and various kinds of diuretics have been used to control hypertension by promoting renal sodium excretion [5], very few studies have focused on intestinal sodium absorption [7], which is the major factor that affects sodium intake. We previously reported that the gastrointestinal tract is a promising initial target for hypertension [8, 9], and that metabolism-related hypertension can be improved by gastric bypass surgery [10]. Further, it has been reported that reducing the sodium content in diet from 150 mmol/day to 100 mmol/day or 50 mmol/day can reduce systolic blood pressure by 2.1 or 4.6 mm Hg [11], respectively; thus, inhibiting intestinal sodium absorption could be beneficial for reducing high blood pressure [7].

The intestine is the main organ involved in sodium and fluid absorption [12]. Na^+ is absorbed by the intestine mainly through Na^+/H^+ exchangers (NHE2/3), Na^+ /glucose-linked transporters, and epithelial Na^+ channels (ENaCs) [13]. Inhibiting intestinal sodium absorption through inhibitors of the Na^+ transporter NHE3, such as SAR218034, results in an increase in fecal sodium excretion and reduction in systolic blood pressure in spontaneously hypertensive rats [14]. In addition to the sodium channels, there are several types of chloride channels present in enterocytes, such as Ca^{2+} -activated Cl^- channel (CaCC) and cystic fibrosis transmembrane conductance regulator (CFTR), which are expressed on the apical or luminal membrane to mediate Cl^- secretion [15–17]. It's reported dietary genistein could regulate basal diabetic jejunum Cl^- secretion in *ob/ob* female mice by reduce CFTR expression [18]. NHE-mediated Na^+ absorption is negatively regulated by CFTR-mediated Cl^- secretion [19]. Moreover, both NHEs and CFTRs are regulated by intracellular second messengers such as cAMP, Ca^{2+} and cGMP; therefore, agents that increase cAMP, Ca^{2+} or cGMP would inhibit electroneutral Na^+ absorption and activate Cl^- secretion [20, 21]. In addition, CFTR also regulates NHE3-mediated Na^+ absorption by altering epithelial cell volume [22]. And Cl^- is able to act as a second messenger for CFTR to modulate the expression of specific genes [23]. Thus, activating intestinal CFTR to promote Cl^- secretion might be an alternative way of inhibiting sodium absorption.

Caffeine (1, 3,7-trimethylxanthine) is one of the most consumed dietary ingredients throughout the world [24, 25]. Caffeine is rapidly absorbed from the gastrointestinal tract and eliminated in 4–6 h [26]. Although acute caffeine intake can raise blood pressure by increasing the activity of the sympathetic nervous system [27], its chronic use lowers blood pressure by promoting renal sodium excretion [28]. Consistent with this, there is new evidence to show that long-term caffeine treatment has a neutral or even beneficial effect on blood pressure, cardiovascular diseases, and heart failure [29, 30]. Further, caffeine has also been shown to be associated with increase in intracellular cAMP levels. This effect is brought about by caffeine-induced activation of ryanodine receptors, which leads to Ca^{2+} release from the Ca^{2+} store and inhibition of phosphodiesterase, and subsequently, increase in the level of

intracellular cAMP [31, 32]. Thus, caffeine could also regulate the activity of NHE3 or CFTR. However, little is known about whether or how caffeine intake regulates sodium absorption.

In this study, we hypothesized that long-term treatment with caffeine decreases Na⁺ absorption by increasing the level of intracellular cAMP in intestinal epithelial cells. Based on the findings obtained, we report that long-term caffeine intake inhibits Na⁺ absorption by intestinal epithelial cells and lowers blood pressure by promoting cAMP-stimulated Cl⁻ secretion through CFTR in salt-sensitive rats.

Materials and Methods

Animal Treatment

Six-week-old male Dahl salt-sensitive rats (Dahl-S, n=20) were obtained from Vital River Company, Beijing, China and housed under controlled temperatures (21–23 °C) with a 12/12 hr light-dark cycle with free access to food and water. After two weeks' adaption, animals were randomly assigned to two groups: The control group (n=10) was given a high salt chow containing 8% (w/w) NaCl and normal drinking water. The caffeine group (n=10) was fed the 8% NaCl chow and 0.1% caffeine in their drinking water. The dietary intervention lasted 15 days. All of the experimental procedures were performed in accordance with protocols approved by Institutional Animal Care and Use Committee of the Institute, and all experiments were performed in accordance with the National Institutes of Health guidelines for the use of experimental animals [33].

Fecal sodium determination

After 15 days of dietary intervention. Rats were put into individual metabolic cages (Tecniplast, Italy) with free access to food and water. The 24 hours' feces were collected. 1 g feces were mixed with 4 ml ultrapure water and bathing in 60°C water for 12 hour and boiling water for 10 minutes. Then the samples were centrifuged at 12000 rpm for 10 minutes. The sodium concentration in supernatant was tested by MI-921 electrolyte analyzer (Yuehua Technology Corporation, Shenzhen, China).

Measurement of NaCl and fluid absorption in the jejunum

The NaCl and fluid absorption function analysis was performed as previous described with some modification [34]. Rats were fasted overnight before the experiment. Rats were anesthetized by inhaling of 2% isoflurane (v/v). Abdomen was opened by one small central incision, a polyethylene tube was advanced into the jejunum (5–6 cm away from stomach), and secured by a ligature that served as an inlet tube. Another polyethylene tube was advanced into distal jejunum and secured by ligature to allow for drainage. The 10–15-cm isolated jejunum segment with an intact blood supply was gently flushed and then perfused at a rate of 6 ml/h with 150 mmol/l NaCl. Effluents from the isolated segment were visually free of blood throughout all experiments. Animals were maintained at 37°C using a heating pad. After an initial 30-min washout and recovery period, fluid absorption was measured for 30min. All values were represented in milliliters of fluid absorbed per centimeter jejunum length per hour (ml/cm/h).

Ussing chamber experiment

After rats were anesthetized with pentobarbital sodium (100 mg/kg body weight ip), the intestine was removed and placed in a cold (4°C) Krebs solution containing (mM): 115 NaCl, 25 NaHCO₃, 2.4 K₂HPO₄, 1.2 CaCl₂, 1.2 MgCl₂, 0.4 KH₂PO₄, and 10mM glucose saturated with 95% O₂-5% CO₂. To prevent exposure of the mucosa to prostanooids during seromuscular stripping, 1 µM indomethacin were added in Krebs solution in advance. The seromusculature layer was carefully removed by fine forceps under stereoscopic microscope. Then the intestine was mounted in Ussing chamber. Tissues were bathed on Krebs solution on both mucosal and serosal side and maintained at 37°C by water-jacketed solution reservoirs. In mucosal side 10mM glucose in Krebs solution was substitute by 10mM mannitol. In some experiment, Cl⁻ free krebs were used and Cl⁻ was substitute by gluconate. All tissues were pretreated (1h) with tetrodotoxin (TTX, 1 µM, serosal) to reduce enteric nerve activity. Tissues were voltage clamped to 0 mV. Short-circuit current (I_{sc}) was continuously recorded on a chart recorder (World Precision Instruments, Inc. Sarasota, FL USA)

and a Power-Lab system 4/26 (AD Instruments, Cattle Hill, Australia). The change in Isc (Δ Isc) induced by a treatment was expressed as the difference from the former baseline to the steady state value.

Chemicals

Caffeine, 4, 4'-Diisothiocyano-2, 2'-stilbenedisulfonic acid (DIDS), 5-Nitro-2-(3-phenylpropylamino) benzoic acid (NPPB), bumetanide, indomethacin, norepinephrine (NE), ruthenium red and clotrimazole were purchased from Sigma Chemical (St. Louis, MO). Tetrodotoxin (TTX) was purchased from Alomone Labs (Jerusalem, Israel). Caffeine, norepinephrine and ruthenium red were dissolved in water. DIDS was dissolved in 0.1 M potassium bicarbonate. NPPB and clotrimazole were dissolved in dimethyl sulfoxide (DMSO), bumetanide was dissolved in ethanol. TTX were dissolved in 0.1M PH 7.4 citrate buffer solution. Drugs were added as a small volume of a stock solution unless otherwise stated.

Statistical analysis

All data represent mean $\pm$ SEM from different rodents. Statistical tests done were: repeated measure analysis and two-way ANOVA followed by the Bonferroni post hoc test to Fig. 1, and two-tailed Student t test to Fig. 2-4. P values < 0.05 indicate statistically significant difference.

Results

Long-term caffeine intervention attenuated salt-sensitive hypertension

Although water and food intake decreased during the first two days of adaptation, chronic caffeine intake did not affect water and food intake in Dahl-S rats over the treatment time that followed (Fig. 1A and 1B). Thus, caffeine intervention did not result in any difference in NaCl intake from the diet. However, long-term caffeine intake attenuated high salt-induced increase in mean arterial blood pressure in a time-dependent manner (Fig. 1C).

Fig. 1. Long-term caffeine intervention attenuated salt-sensitive hypertension. Water intake (A), food intake (B), and mean arterial blood pressure (C) of the control and caffeine-treated rats. Data are shown as the mean $\pm$ SEM values. Significance was calculated using repeated-measures and two-way ANOVA followed by the Bonferroni post-hoc test. *P<0.05 compared with the control group.

Caffeine intake decreased the intestinal absorption of sodium

To investigate whether intestinal sodium absorption plays a role in the anti-hypertensive effect of chronic caffeine intake, we compared sodium absorption in caffeine- and vehicle-treated Dahl-S rats by determining the sodium content in their feces. The results clearly showed that long-term caffeine intake increased the amount of feces (Fig. 2A) and fecal sodium excretion (Fig. 2B) in 24 h. To further explore whether caffeine increased fecal sodium excretion by reducing sodium absorption in the intestine, we analyzed the sodium and water absorptive function of intestine. We perfused the proximal jejunum of rats with isotonic NaCl solution. As NaCl and water were absorbed proportionally in the intestine [13], we monitored the fluid absorption rate as an indicator of sodium absorption (Fig. 2C). We observed that the fluid absorption rate in the caffeine-treated rats was significantly lower than that in the controls; this indicates that long-term caffeine intake reduced sodium absorption in the intestine (Fig. 2D).

Fig. 2. Caffeine intake decreased intestinal absorption of sodium. The 24-h fecal weight (A) and 24-h fecal sodium excretion (B) of the control and caffeine-treated rats. (C) Representative image of the in vivo perfusion experiment. (D) Fluid absorption in the jejunum of the control and caffeine-treated rats. Data are shown as the mean ± SEM values. Significance was calculated using the two-tailed unpaired t-test. *P<0.05 compared with the control group.

Caffeine reduced intestinal Na⁺ absorption by increasing CFTR-mediated Cl⁻ secretion

To explore how caffeine decreased intestinal Na⁺ absorption, we analyzed intestinal ion transportation in rats with the Ussing chamber assay. We found that treatment with caffeine remarkably increased the short circuit current (Isc) in the jejunum mucosa. However, caffeine-induced ΔIsc was almost abolished when Cl⁻ was substituted with gluconate⁻; this could mean that Cl⁻ movement plays a lead role in the caffeine-induced short circuit current (Fig. 3A).

To further explore which channel was responsible for the Cl⁻ secretion, inhibitors of CaCC and CFTR were used. The results showed that DIDS (inhibitor of CaCC) did not affect caffeine-induced ΔIsc, while NPPB (inhibitor of CFTR) almost totally blocked caffeine-induced ΔIsc. In addition, blocking of the Na⁺/K⁺/2Cl⁻ cotransporter by bumetanide in the basement membrane also slightly inhibited caffeine-induced ΔIsc (Fig. 3B). These results confirmed that Cl⁻ movement was the main component of caffeine-induced ΔIsc, and that CFTR was the main effector of the increased Cl⁻ secretion induced by caffeine.

Caffeine activated CFTR by increasing intracellular cAMP

Caffeine acts as an inhibitor of phosphodiesterase and an agonist of the ryanodine receptor, which has the ability to increase the cAMP and Ca²⁺ levels. Both cAMP and Ca²⁺ are important second messengers in Cl⁻ secretion. Thus, to determine whether cAMP was involved in caffeine-induced ΔIsc, we used norepinephrine (NE) to deplete cAMP in jejunum

mucosal cells. After depletion of cAMP by NE, caffeine-induced Δ Isc was almost totally blocked (Fig. 4A). This proves the role of cAMP in caffeine-induced Δ Isc.

To explore whether Ca^{2+} was also involved in caffeine-induced Δ Isc, we used ruthenium red to inhibit the ryanodine receptor. We found that blocking of the ryanodine receptor partly decreased caffeine-induced Δ Isc. Similarly, blocking of the Ca^{2+} -activated potassium channel by clotrimazole also decreased caffeine-induced Δ Isc (Fig. 4B). These results indicate that CFTR activated by cAMP played a major role in caffeine-induced Δ Isc. In addition, K^+ secretion evoked by Ca^{2+} release from the endoplasmic reticulum may also affect caffeine-induced Δ Isc.

Fig. 3. Caffeine reduced intestinal Na^+ absorption through increase in CFTR-mediated Cl^- secretion. (A) Δ Isc in the jejunum mucosa induced by 10^{-4} M caffeine in normal or Cl^- free Kreb's solution in the Ussing chamber. (B) Δ Isc in the jejunum mucosa induced by 10^{-4} M caffeine and DIDS, NPPB, or bumetanide in normal Kreb's solution in the Ussing chamber. Data are shown as the mean $\pm$ SEM values. Significance was calculated using the two-tailed unpaired t-test. * $P < 0.05$ compared with the control group.

Fig. 4. Caffeine activated CFTR by increasing intracellular cAMP. (A) Δ Isc in the jejunum mucosa induced by 10^{-4} M caffeine with or without 10^{-4} M norepinephrine (NE) before caffeine treatment. (B) Δ Isc in the jejunum mucosa induced by 10^{-4} M caffeine with ruthenium red or clotrimazole. Data are shown as the mean $\pm$ SEM values. Significance was calculated using the two-tailed unpaired t-test. * $P < 0.05$ compared with the control group.

Discussion

In the present study, we provide evidence to show that chronic caffeine consumption inhibits NaCl and water absorption by the intestine and reduces blood pressure in Dahl's salt-sensitive hypertension rat model. This effect of caffeine was dependent on reduced electroneutral NaCl absorption by intestinal epithelia cells, via activation of cAMP-stimulated Cl⁻ secretion by CFTR. These results were demonstrated with an *ex vivo* Ussing chamber experiment and an *in vivo* perfusion experiment.

Currently, most of the methods used to counteract high salt intake mainly focus on two strategies—reducing sodium intake and promoting sodium excretion. However, both of them have obvious limitations with regard to their practical application, such as the intolerance of patients to low-sodium food and the side effects of diuretics, which makes it difficult to employ them in daily life. As reported in our previous study, caffeine has a potent anti-hypertensive effect on salt-sensitive hypertension through promotion of urinary sodium excretion [28]. In the present article, we extend the previous findings by reporting that caffeine also possesses a significant inhibitory effect on intestinal sodium absorption. We believe that both its effects on the kidney and intestine contribute to the anti-hypertensive effect of chronic caffeine intake in salt-sensitive animal models; however, it is difficult to determine which effect is more important. Despite this, given these multiple effects of caffeine, it could be an effective alternative intervention option for the treatment of salt-sensitive hypertension that is not limited by dietary restrictions or the side effects of diuretics.

Besides urinary sodium excretion, inhibition of intestinal sodium absorption has been reported to effectively reduce salt-sensitive high blood pressure. For example, inhibition of NHE3 significantly increased fecal sodium excretion from 0.3 to 1.2 g/kg/d in spontaneously hypertensive rats, reduced systolic blood pressure by approximately 20 mm Hg without affecting serum sodium concentration, and also reduced urinary sodium excretion from 1.0 to 0.3 g/kg/d [14]. Further, Anderson et al. reported that caffeine could inhibit NHE3 activity in Caco-2 cells and indirectly inhibit the H⁺-coupled amino acid transporter hPepT1 [35]. In the present study, although the activity of NHE3 could not be detected with the Ussing chamber due to its neutral conductivity, we were able to show that caffeine activated Cl⁻ secretion via CFTR to inhibit electroneutral Na⁺ absorption by NHE3. This implies that reduced NHE3 activity might be involved in the effect of caffeine. However, it is not clear whether the anti-hypertensive effect of caffeine is dependent on reduced NHE3 activity, and this warrants further investigation.

The effect of caffeine on intestinal Na⁺ absorption has been investigated in very few studies. In one such study, Wanger et al. reported that perfusion with caffeine and coffee solutions could lead to net water and sodium secretion in the jejunum compared with the control, but the difference was not significant [36]. Although these results are consistent with the results of the present study, the previous authors did not explore the long-term effects of caffeine intake or the associated mechanisms. Our work clearly indicates that the inhibitory effect of caffeine on sodium absorption is dependent on increased Cl⁻ secretion by CFTR, as use of NPPB, an inhibitor of CFTR, specifically abolished the effect of caffeine on ΔI_{sc}. The active movement of Cl⁻ is as important as Na⁺, and it is also known that the absorption of Na⁺ is inhibited by the secretion of Cl⁻. This means that increase in Cl⁻ secretion could neutralize the electric potential gradient of intestinal epithelial cells, and thereby trigger NHE3 to absorb Na⁺. In addition, both NHE3 and CFTR are regulated by cAMP and intracellular Ca²⁺, which points to the existence of a balance between them. Moreover, the inhibitory effect of cAMP on intestinal Na⁺ absorption is dependent on CFTR. Thus, it is possible that activation of CFTR by caffeine not only directly inhibited NHE3-mediated Na⁺ absorption, but also resulted in the utilization of cAMP and intracellular Ca²⁺ to further reduce NHE3 activity,

which contributed to the increased sodium content in feces. Moreover, as NHE3 and CFTR are also expressed in the kidney [37, 38], the mechanism revealed in the present study might also be involved in caffeine-induced reduction of Na^+ reabsorption in the urine, which was reported in our previous paper.

Until now, the exact role of caffeine in blood pressure regulation remains unclear due to the heterogeneity of previous observational studies and clinical trials. A meta-analysis of randomized controlled trials published in 2005 showed that several studies reported a slight increase in both diastolic and systolic blood pressure in response to coffee or caffeine intake, but the observation period in these studies was relatively short at a range of 7 to 84 days [39]. Another meta-analysis published in 2011 reported an inverse J-shaped dose-response curve for caffeine treatment, in which the hypertension risk increased with consumption of up to 3 cups/day and decreased with higher doses (mean follow-up, 6.4–33.0 years) [40]. Similarly, in another study, an increase in blood pressure was observed several hours after caffeine intake, but long-term (2 weeks) observation showed that there was no increase in blood pressure [29]. If these findings are combined with the present study, it could be deduced that the anti-hypertensive effect of caffeine depends on a relatively long treatment period and a higher dosage. Therefore, a longer observation time and treatment with a gradient concentration of caffeine may be useful for obtaining a more reliable conclusion.

Overall, the data presented here support the hypothesis that caffeine regulates salt-sensitive hypertension by controlling intestinal sodium absorption. Further, this is the first study to present Cl^- and CFTR as potential molecular targets of caffeine in the intestine via which it could decrease salt-sensitive hypertension. The results further reveal that increased Cl^- secretion by CFTR accounts for the reduction in intestinal Na^+ absorption induced by caffeine. This work emphasizes the importance of reduction in intestinal sodium absorption as an effective and feasible strategy for the prevention of salt-sensitive hypertension.

Disclosure Statement

The authors declare no conflict of interest.

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