Effect of Sairei-to on signal transduction of the vasopressin V2 receptor in rat renal cortex

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Sairei-to (TJ-114), a traditional Japanese medicine, has been used clinically for the treatment of various edematous disorders. The inhibitory effect of TJ-114 on edema may be dependent on the diuretic response it invokes. The present study was performed to determine the effect of TJ-114 on the anti-diuretic hormone vasopressin, which is implicated in the retention of water in various edematous disorders. TJ-114 (0.5-1.5 g/kg) was administered intra-duodenally to pentobarbital-anesthetized rats. Specimens of the renal cortex were isolated 30 min after the administration and incubated in buffered Hank’s balanced salt solution with vasopressin. The vasopressin-stimulated cAMP production was dose-dependently attenuated in renal tissues in rats treated with TJ-114. The inhibitory effect of TJ-114 was diminished by pre-treatment with N\textsuperscript{G}-nitro-L-arginine methyl ester, a nitric oxide synthase inhibitor. Therefore, TJ-114 may inhibit stimulation of the vasopressin V2 receptor which is closely related to nitric oxide production.

Key words Sairei-to, vasopressin, nitric oxide, diuresis.
Abbreviations NO, nitric oxide; L-NAME, N\textsuperscript{G}-nitro-L-arginine methyl ester; cAMP, adenosine 3’, 5’-cyclic monophosphate.

Introduction

Kampo medicine has been used empirically in the treatment of various edematous disorders. Sairei-to (TJ-114) is composed of 12 crude drugs (Table 1) and has been reported to be effective in the treatment of edema accompanying nephrosis, cirrhosis or pregnancy, macular edema, swelling and lymphedema after surgery.\textsuperscript{1-3} In general, an enhanced diuresis is considered useful for the treatment of edema with characteristics of sodium and water retention. It has been demonstrated that Sairei-to increased urine volume in rats\textsuperscript{6} and mice,\textsuperscript{7} suggesting that the beneficial effect on edema is dependent on the diuretic response to Sairei-to.

Ohnishi et al. reported that Sairei-to increased urine volume in water-overloaded mice produced by a single intraperitoneal pretreatment with desmopressin acetate, an anti-diuretic hormone vasopressin V2 receptor analog, followed by an injection of physiological saline.\textsuperscript{7} Its mechanism of action, however, is not yet fully understood. Our previous experiment suggested that the stimulation of nitric oxide (NO) production contributes to the diuretic effect of Sairei-to in pentobarbital-anesthetized rats.\textsuperscript{8} NO plays a role not only in the regulation of systemic circulation as a potent vasodilator, but also in various physiological processes in the kidney. It has been demonstrated that NO modulates renal circulation, sodium transport in several tubule segments of nephrons, and vasopressin-stimulated water permeability in collecting ducts. Thus, we investigated the effect of Sairei-to on the signal transduction system for vasopressin and the association with NO in rat renal cortex ex vivo.

Materials and Methods

Animals and Drugs. Male Wistar rats were obtained from SLC (Hamamatsu, Japan). They were housed in a cage and allowed free access to water and standard laboratory food (MF, Oriental Yeast, Tokyo, Japan). All experimental procedures were performed according to the "Guidelines for the Care and Use of Laboratory Animals" approved by the Laboratory Animal Committee of Tsumura & Co.

Tsumura Sairei-to (TJ-114) is composed of 12 crude drugs in fixed proportions (Table 1). It was prepared as a spray-dried powder from a hot-water extract. N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME), a NO synthase inhibitor, and [Arg8]-vasopressin were purchased from Sigma (St. Louis, MO USA).

Experimental procedure. Rats fasted overnight were anesthetized by intraperitoneal injection of sodium pentobarbital, 50 mg/kg body weight. The duodenum was cannulated with polyethylene tubing (PE 50) for the drug administration. Sairei-to (0.5, 1.0 or 1.5 g/kg) prepared in distilled water and the vehicle (5mL/kg water) were administered to rats (n=6-9). In the other set of experiments (n=8-9), the interaction with L-NAME in the Sairei-to (1.5 g/kg)-treated rats was examined. L-NAME (6 mg/kg, i.p.) was injected 5 min before the administration of Sairei-to or the vehicle.

Measurement of vasopressin-stimulated cAMP production. At 30 min after the administration of Sairei-to, specimens of renal cortex were isolated in a Petri dish on ice, then finely sliced in order to condition them in buffered Hank’s balanced salt solution (HBSS), pH 7.4. After the
Table 1 Composition of Sairei-to (TJ-114)

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupleuri radix, Bupleurum falcatum LINNE (Hebei province, 河北省; China)</td>
<td>7.0 g</td>
</tr>
<tr>
<td>Pinelliae tuber, Pinellia ternata BREITENBACH (Sichuan prov., 四川省; China)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Alismatis rhizoma, Alisma orientale JUZECZUK (Sichuan prov., 四川省; China)</td>
<td>5.0 g</td>
</tr>
<tr>
<td>Scutellariae radix, Scutellaria baicalensis GEORGII (Hebei prov., 河北省; China)</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Ginseng radix, Panax ginseng C. A. MEYER (Jilin prov., 吉林省; China)</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Zizyphi fructus, Zizyphus jujuba MILLER var. inermis REHDER (Hebei prov., 河北省; China)</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Polyoporus, Polyporus umbellatus FRIES (Hubei prov., 湖北省; China)</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Atractylodis lancea rhizoma, Atractylodes lancea DE CANDOLLE (Hubei prov., 湖北省; China)</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Poria, Poria cocos WOLF (Yunnan prov., 云南省; China)</td>
<td>3.0 g</td>
</tr>
<tr>
<td>Glycyrrhiza radix, Glycyrrhiza uralensis FISCHER (Jilin prov., 吉林省; China)</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Cinnamomi cortex, Cinnamomum cassia BLUME (Guangxi prov., 广西省; China)</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Zingiberis rhizoma, Zingiber officinale ROSCOE (Sichuan prov., 四川省; China)</td>
<td>1.0 g</td>
</tr>
</tbody>
</table>

weighed sliced tissue had been placed in 130 μL of HBSS gassed with 95% O₂ and 5% CO₂ in a microtube, 50 μL of 5 mmol/L 3-isobutyl-1-methylxanthine (IBMX) in HBSS was added. The samples were incubated for 3 min at 37 °C. [Arg]-vasopressin (final concentration, 1 and 10 mIU/mL) was then added to bring the final volume of each sample to 200 μL. The samples were incubated for an additional 2 min at 37 °C. At the end of this incubation, the tube was placed on ice, and 200 μL of ice-cold HBSS containing 1.25 mmol/L IBMX was added immediately to each sample.

Subsequent extraction and analysis of adenosine 3′, 5′-cyclic monophosphate (cAMP) was conducted with an cAMP EIA kit (No.RPN 225) from Amersham Life Science (Little Chalfont, UK). An aliquot of 100 μL of ice-cold 10% trichloroacetic acid (TCA), which was diluted in 50 mmol/L sodium acetate buffer (pH 5.0), was added to the renal cortical tissue removed from the incubation buffer. Each sample was placed on ice for 5 min and then homogenized in an immersion sonicator. The samples were centrifuged at 10,000 rpm for 10 min, and the supernatant was removed of TCA by extraction with water-saturated diethyl ether. The amount of cAMP in tissue extracts was determined by the acetylation assay procedure (range 2-128 fmol/well), as described in the kit’s directions.

Statistical analysis. Data were expressed as the mean ± S.E.M. The statistical differences between groups were evaluated using a one-way analysis of variance (ANOVA) followed by Dunnett’s test or Fisher’s PLSD test. Differences were considered to be statistically significant if p<0.05.

Results

As shown in Figure 1, the level of cAMP in the renal cortical tissues was increased by the incubation with 1 and 10 mIU/mL of vasopressin. In comparison with the control group, a dose-dependent attenuation of vasopressin-stimulated cAMP production was observed in renal tissues on administration of Sairei-to.

As shown in Figure 2, basal levels of cAMP before stimulation of vasopressin in renal cortical tissues were not changed by the administration of Sairei-to. However, these

![Fig. 1](image1.png) Effect of TJ-114 on arginine vasopressin (AVP)-stimulated cAMP production in renal cortical tissue. At 30min after the administration of TJ-114 or distilled water in pentobarbital-anesthetized rats, specimens of renal cortex were isolated and incubated in the presence of 1 or 10mIU/mL AVP. *p<0.05, **p<0.01, significant difference compared with the value for distilled water-treated rats (n=6-9) (Dunnett’s test).

![Fig. 2](image2.png) Basal levels of cAMP in renal cortex tissue. Specimens of renal cortex were isolated at 30min after the administration of TJ-114 (1.5 g/kg) or distilled water (DW) in pentobarbital-anesthetized rats. L-NAME (6 mg/kg) or saline was injected i.p. 5 min before the administration of TJ-114 or DW. *p<0.05, significant difference compared with DW(-) (n=8-9) (Fisher’s PLSD test).
levels tended to increase following administration of L-NAME with or without Sairei-to. As shown in Figure 3, pre-intraperitoneal injection of L-NAME did not alter vasopressin-stimulated cAMP production in vehicle-treated rats, however, it significantly diminished the inhibition of cAMP production in Sairei-to-treated rats.

Discussion

In the present study, it was clarified that the increase in cAMP production stimulated by vasopressin in the renal cortex was dose-dependently attenuated on administration of Sairei-to in rats. Moreover, the effect of Sairei-to was diminished by pre-treatment with L-NAME. These findings suggest that Sairei-to modulates the action of vasopressin in rat renal tissues, which may be mediated by the activation of NOS.

Vasopressin, an anti-diuretic hormone, is known to play a major role in water metabolism by inducing the reabsorption of water through stimulation via V2 receptors at renal collecting ducts. It has been demonstrated that V2 receptors are coupled to the adenylyl cyclase-cAMP-protein kinase A pathway, and phosphorylated aquaporin-2, a water channel. It has been reported that vasopressin-stimulated water permeability is counter-regulated by several other hormones/autocoids including atrial natriuretic factor, prostaglandin E2 and dopamine. In addition, using isolated perfused cortical collecting ducts in vitro, Garcia et al. have shown that NO inhibited vasopressin-stimulated water permeability, and mediated activation of cGMP-dependent protein kinase, which in turn decreases intracellular cAMP levels.

In the present study, the basal levels of cAMP tended to increase in renal tissues after administration of L-NAME. It was considered that this may have been induced by the activation of adenylyl cyclase due to inhibition of NO production in the renal tissues. Sairei-to significantly attenuated the vasopressin-stimulated cAMP production, and this effect was inhibited by pre-treatment with L-NAME. Our previous report demonstrated that Sairei-to enhanced urine volume and urinary excretion of NO metabolites (NO2 + NO3) in pentobarbital-anestheitized rats, and the inhibition of these effects by L-NAME suggested that Sairei-to activated endogenous NO production. Therefore, the inhibitory effect of Sairei-to on vasopressin-stimulated cAMP production may be mediated by NO production. In the present study, the basal level of cAMP was not decreased in renal tissues after administration of Sairei-to. Awazu et al. has reported that Sairei-to increases the generation of cAMP and inhibits proliferation of cultured rat mesangial cells by suppressing Raf-1/ERK cascade. Thus, it is possible that the basal level of cAMP in renal tissues after administration of Sairei-to is affected by other NO-independent mechanisms.

It has been reported that NO stimulates vasopressin secretion in the posterior pituitary gland. These findings suggest that NO may participate in the down-regulation of vasopressin V2 receptor in renal tissue. There is no evidence regarding the effects of Sairei-to or L-NAME on the level of vasopressin V2 receptor in the present study. Further studies are needed to better understand the mechanism by which Sairei-to exerts its effect.

We have demonstrated that Sairei-to is useful for the suppression of edema in anti-glomerular basement membrane nephritic mice. Recent reports show that a nonsomatic vasopressin release is implicated in the water retention of various edematous disorders. The effectiveness of Sairei-to against edematous disorders is probably associated with the inhibition of the anti-diuretic action of vasopressin.

Several reports have demonstrated that the blocking of sodium channel may participate in the mechanism of diuretic action of Sairei-to. The effect of Sairei-to on the action of vasopressin in this experiment is a new finding. Gorei-san is also known to increase urine volume in water-overloaded mice, however, its action mechanism is not yet fully understood. It has been reported that Alismatis rhizoma, which is a common component of Sairei-to and Gorei-san, could improve platelet aggregation in diabetes through increased production of nitric oxide. Alismatis rhizoma may be an important component of the diuretic effects of Sairei-to.

In conclusion, we have demonstrated that Sairei-to inhibits vasopressin-stimulated cAMP production in rat renal tissues probably by activating NOS. These effects may partially contribute to the diuretic effect of Sairei-to.
References


Japanese abstract

柴苓湯は臨床上の様々な浮腫に用いられており、その効果は本剤の利尿作用によるところが少なくないと考えられてい る。しかし、柴苓湯の利尿作用機序についてはまだ不明な点が多い。今回、抗利尿ホルモン（vasopressin）の刺激に対する柴苓湯の作用について検討した。雄性Wistarラットに柴苓湯（0.5〜1.5g/kg）を投与し、10分後に採取した腎皮質切片を用い、ミクロスコープ下で観察した。その結果、柴苓湯の利尿作用は柴苓湯投与群が得た腎皮質において用による量依存的に抑制され、さらに柴苓湯のcAMP産生抑制作用は一酸化窒素合成酵素阻害薬であるL-NAME（6mg/kg、p.o.）の前処置によって消失した。柴苓湯は一酸化窒素の産生を増加させることでcAMPのV2受容体が刺激された以降の細胞内情報伝達を遮断する可能性が示唆された。これらの結果から、柴苓湯の利尿作用の一部はAVPが関与しているかもしれない。

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