Effect of naftopidil on brain noradrenaline-induced decrease in arginine-vasopressin secretion in rats

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A B S T R A C T

Naftopidil, an α1-adrenoceptor antagonist, has been shown to inhibit nocturnal polyuria in patients with lower urinary tract symptoms (LUTS) owing to its effects in reducing resistance in the prostatic urethra (1). In male patients with LUTS, naftopidil has also been shown to be effective against nocturia (2). Furthermore, Yokoyama et al. (3) reported that nocturnal polyuria in male patients with LUTS significantly decreased upon naftopidil administration, indicating that it can directly reduce nocturnal urine production. Recently, another study found that naftopidil can cross the blood–brain barrier to easily enter the central nervous system (4). However, the mechanism by which naftopidil decreases nocturnal urine production has still not been well understood.

Keywords: Naftopidil Arginine-vasopressin Noradrenaline Brain Nocturnal polyuria

1. Introduction

Naftopidil is an α1-adrenoceptor antagonist that used in the treatment of benign prostatic hyperplasia (BPH)-associated lower urinary tract symptoms (LUTS) owing to its effects in reducing resistance in the prostatic urethra (1). In male patients with LUTS, naftopidil has also been shown to be effective against nocturia (2). Furthermore, Yokoyama et al. (3) reported that nocturnal polyuria in male patients with LUTS significantly decreased upon naftopidil administration, indicating that it can directly reduce nocturnal urine production. Recently, another study found that naftopidil can cross the blood–brain barrier to easily enter the central nervous system (4). However, the mechanism by which naftopidil decreases nocturnal urine production has still not been well understood.

Arginine vasopressin (AVP), an antidiuretic hormone, is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and plays an important role in the maintenance of serum osmolality and volume through free water excretion. The release of AVP into the plasma, induced by both osmotic and non-osmotic stimuli, is modulated by brain adrenoceptors (5). In healthy adults, diurnal secretion of AVP into peripheral blood peaks during nighttime (6), and is regulated by the circadian rhythm. However, patients with nocturnal polyuria do not show significantly elevated plasma AVP levels at nighttime, suggesting that abnormal diurnal variation in AVP secretion is highly prevalent in these patients (7,8).

Spontaneously hypertensive rats (SHRs) are a valuable tool for exploring the pathogenesis of hypertension-related bladder dysfunction. These rats exhibit increased voiding frequency and decreased bladder blood flow compared to the non-hypertensive Wistar rats (9,10). Saito et al. (11) revealed that naftopidil decreases micturition frequency and urine production in SHRs during the light-cycle. It has been reported that basal and K+-stimulated release of endogenous noradrenaline (NA) from the paraventricular hypothalamic nucleus was increased in SHRs compared with
normotensive control rats, suggesting that noradrenergic neuronal activity is enhanced in the central nervous system of SHRs (12). Furthermore, inhibition of NA synthesis in the posterior hypothalamus by 6-hydroxydopamine was demonstrated to lower blood pressure in SHRs (13).

Based on these reports, we postulated that naftopidil either directly or indirectly regulates AVP secretion via brain adrenoceptors, leading to reduction in urine frequency and production at night. In the present study, we examined whether naftopidil modulates plasma AVP levels and urine production in rats centrally administered with NA.

2. Materials and methods

2.1. Animals

All animal care and experimental procedures complied with the guiding principles for care and use of laboratory animals approved by Kochi University (No. 1-00046), in accordance with the “Guidelines for proper conduct of animal experiments” proposed by the Science Council of Japan; these guidelines conform to the standards of the National Institutes of Health. All efforts were made to minimize the suffering of the animals and the number of animals needed to obtain reliable results. In all, 93 eight-week-old male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) weighing 200–250 g were used in the experiments. The rats were housed in pairs in cages, in an air-conditioned room at 22–24 °C under a constant day–night rhythm (14 h light–dark cycle. lights on at 05:00) for more than 2 weeks. They had ad libitum access to food (laboratory chow, CE-2; Clea Japan, Hamamatsu, Japan) and water. Upon reaching a body weight of 310–360 g, the rats were subjected to the following experiments.

2.2. Intracerebroventricular administration of NA

In the morning (10:30–11:30), rats were placed in a stereotaxic apparatus (Narishige, Tokyo, Japan) under urethane anesthesia (1.0 g/kg, i.p.), as described previously in a published work of this laboratory (14). The skull was drilled for intracerebroventricular administration of NA using a stainless-steel cannula (outer diameter of 0.3 mm). The stereotaxic coordinates of the tip of the cannula were as follows (in mm): AP −0.8, L 1.5, V 4.0 (AP, anterior from the bregma; L, lateral from the midline; V, below the surface of the brain), according to the rat brain atlas (15). The steel cannula was injected into the left lateral ventricle 3 h before NA administration as described below, and was retained until the end of the experiment.

2.3. Drug administration

NA and naftopidil were dissolved in 10 mM phosphate-buffered saline (PBS) containing 0.1% ascorbic acid (pH 7.4) and 10% N,N-dimethylformamide (DMF), respectively. NA (3 or 30 µg/kg) was slowly administered into the left lateral ventricle in a volume of 10 µL per animal using a cannula connected to a 10-µL Hamilton syringe (Hamilton, Reno, NV, USA) at a rate of 10 µL/min. Naftopidil (10 or 30 mg/kg) was administered intraperitoneally in a volume of 1.0 mL per animal. Subsequently, NA was slowly administered as described above, 3 h following the application of naftopidil. The doses of naftopidil were determined according to previous reports from our and other laboratories that had used similar doses of naftopidil in rats (4,11,16). Furthermore, we have checked the dose-dependent efficacy of naftopidil in our preliminary study. The exact location of the cannula injected in the brain was confirmed at the end of each experiment by verifying that cresyl violet, injected through the cannula, had spread throughout the ventricular system, as described previously (17). For voiding behavior studies, rats were administered naftopidil intraperitoneally at 10:00 am once a day, for two days.

2.4. Measurement of plasma AVP levels

A total of 70 rats placed in a stereotaxic apparatus were randomly divided into ten groups in order to measure their plasma AVP levels: NA administered groups at 3 and 30 µg/kg per animal, i.c.v. (n = 7 and 8, respectively); vehicle-1 (10 µL PBS-containing 0.1% ascorbic acid per animal, i.c.v.) administered group (n = 7); naftopidil administered groups at 10 and 30 mg/kg, i.p. (n = 8 and 7, respectively); vehicle-2 (1.0 mL 10% DMF, i.p.) administered group (n = 6); 10 mg/kg naftopidil (i.p.) and NA (30 µg/kg, i.c.v.) administered group (n = 8); 30 mg/kg naftopidil (i.p.) and NA (30 µg/kg, i.c.v.) administered group (n = 6); vehicle-2 and NA (30 µg/kg, i.c.v.) administered group (n = 7); and vehicle-2 and vehicle-1 administered group (n = 6).

Blood samples (5 mL) were collected from the inferior vena cava 1 h after NA or vehicle administration into the left ventricle. All blood samples were collected at 4:00 pm as described previously (11), and were mixed with 1 mL EDTA, centrifuged at 1600 × g for 15 min at 4 °C to obtain the plasma. The obtained plasma samples were mixed with a protease inhibitor cocktail (0.5 µL/mL plasma), and were kept at −80 °C. These samples were concentrated to 400 µL using a centrifugal concentrator, before AVP levels were measured in duplicate using an ELISA kit (#ab133028; Abcam, Cambridge, UK). The sensitivity of this assay (lower limit of detection) was less than 3.39 pg/mL, and the intra-assay precision was less than 5.9%.

2.5. Voiding behavior studies

Rats were randomly divided into two groups for examination of voiding behaviors: naftopidil (30 mg/kg, i.p.) administered group (n = 5) and vehicle-2 administered group (n = 5). Voiding behavior studies were performed according to methods described in our previous reports (11). The rats received food and water ad libitum from the time they were initially placed in metabolic cages. The rats were kept for 24 h for adaptation, and recorded for the next 24 h. Micturition frequency and total urine output were evaluated from these recordings.

2.6. Urine production and osmolality

Fourteen rats were divided into the following groups: NA (30 µg/kg, i.c.v.) administered group (n = 5); NA (30 µg/kg, i.c.v. and naftopidil (30 mg/kg, i.p.) administered group (n = 5); and vehicle-1 and vehicle-2 administered group (n = 4). Before the experiment, rats were catheterized at the bladder dome with a 22G needle for urine collection, and a stainless cannula was inserted into the left lateral ventricle for administration of NA as described above. The catheterized bladder in the rat was emptied and the urethra was clamped to prevent urine leakage. Vehicle-1 or NA (30 µg/kg, i.c.v.) was centrally administered 3 h after intraperitoneal pretreatment with vehicle-2 or naftopidil (30 mg/kg). Urine was collected 1 and 3 h after NA administration (at 4:00 pm and 6:00 pm, respectively).

We also determined urine osmolality for the following groups: NA (30 µg/kg, i.c.v.) administered group (n = 5); NA (30 µg/kg, i.c.v.) and naftopidil (30 mg/kg, i.p.) administered group (n = 5); and vehicle-1 and vehicle-2 administered group (n = 5). Urine was collected 1 and 3 h after NA administration (at 4:00 pm and 6:00 pm, respectively), and was centrifuged at 1600 × g for 15 min at 4 °C to remove impurities. The urine osmolality (mOsmolality/kg H2O) of...
the supernatant was measured by means of an osmometer (OM-2030; Aryray, Kyoto, Japan) based on the freezing point method (19).

2.7. Statistical analysis

Data are expressed as mean ± SEM. Comparisons between two or more groups were performed by unpaired t-test or analysis of variance (ANOVA) followed by Bonferroni post hoc test, respectively. Differences with a p-value of 0.05 or less are considered statistically significant.

2.8. Drug and chemicals

Urethane, NA, and protease inhibitor cocktail were purchased from Sigma Aldrich (St Louis, MO, USA). Naftopidil was purchased from Tokyo Chemical Industry (Tokyo, Japan). All other chemicals were from Nacalai Tesque (Kyoto, Japan), except stated otherwise.

3. Results

3.1. Effect of centrally administered NA on plasma level of AVP

We first investigated whether centrally administered NA affects plasma levels of AVP. It has been reported that AVP levels in plasma and in the brain exhibit marked changes during light- and dark-cycles (20). Furthermore, Greeley et al. (18) revealed that maximum plasma AVP levels (approximately 13–18 pg/mL) were observed during the midafternoon through the early evening in rats. As shown in Fig. 1, treatment with vehicle-1 had no effect on the basal plasma levels of AVP (19.2 ± 4.0 pg/mL), which were consistent with the observations of Greeley et al. (18) and other laboratories (21,22). On the other hand, NA (3 and 30 μg/kg/animal, i.c.v.) dose-dependently decreased plasma AVP levels 1 h after its administration (Fig. 1).

3.2. Effect of systemically administered naftopidil on plasma level of AVP

To evaluate whether naftopidil by itself affects the plasma level of AVP, rats received i.p. injections of naftopidil. As shown in Fig. 2, there were no significant differences in plasma AVP level between rats 4 h after treatment with vehicle-2 or naftopidil.

3.3. Effect of systemically administered naftopidil on the centrally administered NA-induced decrease in plasma level of AVP

Next, we investigated whether naftopidil affects the NA-induced reduction in plasma AVP level. Rats were pretreated with vehicle-2 or naftopidil (10 and 30 mg/kg, i.p.) 3 h before NA administration (30 μg/kg, i.c.v.). Administration of naftopidil at a higher dose effectively abolished the NA-induced decrease in plasma AVP level (Fig. 3).

3.4. Voiding behaviors in rats systemically administered with naftopidil

Results of the voiding behavior studies are shown in Table 1. Basal levels of urine volume in rats treated with vehicle-2, collected during light- and dark-cycles, were 8.4 ± 0.5 and 12.6 ± 2.2 mL, respectively. Treatment with 30 mg/kg naftopidil (i.p.) produced no significant difference in urine volume between rats administered with naftopidil (9.8 ± 1.0 mL during light-cycle, 12.1 ± 1.5 mL during dark-cycle) and vehicle-2, regardless of the light- and dark-cycle. Similarly, basal levels of urinary frequency in the vehicle-treated rats (7.0 ± 0.9 and 11.5 ± 1.2 times during light- and dark-cycles, respectively) were not significantly affected by naftopidil treatment (5.4 ± 0.2 times during light-cycle, 8.6 ± 1.6 times during dark-cycle).

3.5. Effect of systemically administered naftopidil on urine production and osmolality in rats centrally administered with NA

To investigate whether naftopidil affects urine production and its osmolality in rats treated with NA, urine was collected at 1 and 3 h after NA administration (Table 2). Compared to the vehicle-1 and -2 treated control group, NA administration resulted in a significant increase in urine volume that remained for at least 3 h after its administration. Remarkably, the increase in urine volume 3 h after NA administration was almost completely suppressed when rats were pretreated with naftopidil (30 mg/kg, i.p.) 3 h before NA administration. In contrast, there were no

Fig. 1. Effect of centrally administered NA on plasma level of AVP. Blood samples were obtained from the inferior vena cava 1 h after treatment with vehicle or NA (3 or 30 μg/kg, i.c.v.). Results are presented as mean ± SEM. * p < 0.05 vs. control rats treated with vehicle.

Fig. 2. Effect of naftopidil on plasma level of AVP. Blood samples were obtained from the inferior vena cava 4 h after treatment with vehicle or naftopidil (10 or 30 mg/kg, i.p.). Naf: naftopidil. Results are presented as mean ± SEM.

Fig. 3. Effect of naftopidil on the NA-induced decrease in plasma level of AVP. Rats were treated with vehicle or NA (30 μg/kg, i.c.v.) 3 h after pretreatment with vehicle or naftopidil (10 or 30 mg/kg, i.p.). Blood samples were obtained from the inferior vena cava 1 h after NA administration. Results are presented as mean ± SEM. * p < 0.05 vs. control rats treated with vehicle. Naf: naftopidil. # p < 0.05 vs. NA-treated rats, pretreated with vehicle or 10 mg/kg naftopidil.
significant differences in urine volume collected at 1 h after NA administration between rats untreated and pretreated with naftopidil. Urine osmolality decreased significantly 1 h after NA treatment compared to control rats. However, there were no significant differences in urine osmolality collected 3 h after NA treatment. Although the decrease in urine osmolality was suppressed by naftopidil pre-administration, it was not statistically significant.

4. Discussion

In this study, we demonstrated that 1) centrally administered NA significantly decreased plasma level of AVP in a dose-dependent manner; 2) a decrease in plasma AVP level in rats induced by NA was dose-dependently suppressed by pre-administration of naftopidil; and 3) while naftopidil suppressed NA-induced urine production in rats, it had no effect on the urine production in normal condition. These findings suggest a novel mechanism by which naftopidil ameliorates nocturnal polyuria.

α1-Adrenoceptor antagonists are the most common drugs used in the treatment of male LUTS suggestive of BPH, which act via the relaxation of prostatic smooth muscles. Tamsulosin, an α1-adrenoceptor antagonist, improves the maximum urinary flow rate and the frequency of nocturia, without decreasing nocturnal urine volume in BPH patients (23). Similarly, terazosin, another such an antagonist, also reduces the frequency of nocturia in BPH patients despite there being no detectable reduction in nocturnal urine volume (24). In limited countries including Japan, China, and South Korea, naftopidil was administratively approved for the treatment of BPH and BPH-associated LUTS (25). A clinical study comparing tamsulosin and naftopidil by Nishino and colleagues (26) demonstrated that while both drugs had similar efficacy against BPH/LUTS, naftopidil was more effective against nocturia than tamsulosin. Furthermore, naftopidil has been reported to decrease urine production and the frequency of voids at nighttime, regardless of incidences of sleep disturbance (3). Recently, we revealed that naftopidil decreases micturition frequency and urine production during the light-cycle in a rat model of hypertension-related bladder dysfunction (11). It seemed that naftopidil either directly or indirectly increased the secretion of AVP in rats, thus contributing to the inhibition of urine production during sleep. In the current study, we showed that naftopidil suppressed the increase in urine production in rats centrally administered with NA, but did not affect urine production in rats untreated with NA, during both light- and dark-cycles. Recently, naftopidil has been reported to be able to cross the blood–brain barrier and act on several regions in the central nervous system as well as the bladder (4,27,28). Therefore, the effects of naftopidil on urine production may be attributed to a direct inhibition of dysregulated NA activity in the central nervous system, while, in this study, we did not examine whether central administration of other α1-adrenoceptor antagonists such as tamsulosin and terazosin suppresses the NA-induced increase in urine production in rats.

NA is an important neurotransmitter in the supraoptic nucleus, and noradrenergic projection from the locus coeruleus is known to promote wakefulness in the hypothalamus (29). In a recent study involving a rat model of sleep disturbance induced by repeated administration of corticosterone, NA level was found to be markedly increased in the locus coeruleus and hypothalamus (30). On the other hand, improvement in sleep disturbance has been reported to decrease the frequency of nocturia, accompanied by reduction in nocturnal polyuria in the elderly (31). Thus, we considered the NA-induced increase in urine production in our study as a model of nocturnal polyuria. This was supported by our findings that systemic injection of naftopidil suppressed the increase in urine production. However, little is known about the relationship between noradrenergic neuronal activation in the central nervous system and nocturnal polyuria. Therefore, further studies are required to determine whether the former contributes to the latter.

Table 1

<table>
<thead>
<tr>
<th>Urinary volume (mL)</th>
<th>Light-cycle</th>
<th>Dark-cycle</th>
<th>Whole-cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>8.4 ± 0.5</td>
<td>12.6 ± 2.2</td>
<td>21.1 ± 1.9</td>
</tr>
<tr>
<td>Naf</td>
<td>9.8 ± 1.0</td>
<td>12.1 ± 1.5</td>
<td>21.9 ± 2.4</td>
</tr>
<tr>
<td>p-value</td>
<td>0.242</td>
<td>0.844</td>
<td>0.800</td>
</tr>
</tbody>
</table>

Vehicle: Wistar rats treated with 10% DMF (i.c.v.).
Naf: Wistar rats treated with naftopidil (30 mg/kg, i.p.).
p-Value: Vehicle-administered group vs. naftopidil-administered group.
Data are shown as mean ± SEM.

Table 2

<table>
<thead>
<tr>
<th>Urinary volume (mL)</th>
<th>1 h</th>
<th>3 h</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>0.5 ± 0.0</td>
<td>0.8 ± 0.0</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>Naf</td>
<td>0.9 ± 0.1*</td>
<td>1.5 ± 0.2*</td>
<td>2.5 ± 0.2*</td>
</tr>
<tr>
<td>Naf + NA</td>
<td>0.7 ± 0.1</td>
<td>0.7 ± 0.0*</td>
<td>1.4 ± 0.0*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urinary osmolality (mOsmolality/kgH2O)</th>
<th>1 h</th>
<th>3 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>1965.4 ± 156.2</td>
<td>1964.5 ± 240.4</td>
</tr>
<tr>
<td>Naf</td>
<td>1498.0 ± 97.4*</td>
<td>1719.7 ± 159.6</td>
</tr>
<tr>
<td>Naf + NA</td>
<td>1748.2 ± 145.9</td>
<td>1941.0 ± 77.3</td>
</tr>
</tbody>
</table>

Vehicle: Wistar rats treated with DMF (i.c.v.) 3 h after pretreatment with PBS (i.p.).
NA: Wistar rats treated with NA (30 μg/kg, i.c.v.) 3 h after pretreatment with PBS (i.p.).
Naf + NA: Wistar rats treated with NA (30 μg/kg, i.c.v.) 3 h after pretreatment with naftopidil (30 mg/kg, i.p.).
1 h: the volume of urine that was collected 1 h after NA treatment (6:00 pm).
3 h: the volume of urine that was collected 3 h after NA treatment (9:00 pm).
Total: the total volume of urine that was collected 1 and 3 h after NA treatment.
Data are shown as mean ± SEM.

*Significantly different from the Vehicle group (p < 0.05).
*Significantly different from the NA group (p < 0.05).
Previously, we and other laboratories have evaluated the effects of naftopidil on urine production in both basic and clinical studies (31,11). While there is no information available that demonstrates the mechanisms by which naftopidil directly reduces urine production, it has been reported that naftopidil improves bladder capacity by suppressing C-fiber afferent activity (32). In the present study, we found that naftopidil suppressed the decrease in plasma AVP level in rats centrally administered NA. There are several reports demonstrating that injection of NA into the cerebral ventricles excites AVP-containing cells and stimulates release of AVP (5,33); nonetheless, NA has also been reported to inhibit AVP release (16,34). In general, activation of α2-adrenoceptor excites neurons in the supraoptic nucleus and stimulates AVP release, while β-adrenoceptor activation inhibits these neurons (35). It has been suggested that the inhibitory effect of NA on AVP release may be mediated by α2-adrenoceptors, as the infusion of clonidine, an α2-adrenoceptor agonist, into the lateral cerebral ventricle markedly decreased the release of AVP in anesthetized dogs (36). Since these effects of the different adrenoceptor subtypes on AVP secretion are not consistent with the antagonistic specificity of naftopidil, it is necessary to clarify which subtype of adrenoceptor is involved in the action of naftopidil in future investigations. To our knowledge, this is the first report demonstrating that the beneficial effect of naftopidil against NA-induced polyuria may be mediated by its ameliorating action on NA-induced dysregulation in AVP secretion from vasopressin-containing neurons into peripheral blood. Furthermore, we found that central administration of NA resulted in a significant reduction in urine osmolality. These results suggested that the observed changes in urine volume were dependent on the renal action of AVP.

In the present study, we utilized rats that have been acclimated for two weeks on a 14/10-h light–dark cycle according to the breeding condition proposed by our Institute for Animal Research, while other researchers have adopted a 12/12-h light–dark cycle to investigate plasma AVP level in rats (37,38). Since the rhythm of AVP secretion is maintained under dark conditions (39), we cannot rule out the possibility that our particular light–dark cycle affected the circadian change of plasma AVP level. Therefore, further investigations are necessary to clarify the mechanism by which naftopidil inhibits the reduction in plasma AVP level in rats centrally administered with NA.

5. Conclusions

In summary, systemic pre-administration of naftopidil suppressed the decrease in plasma AVP level and the increase in urine production in rats centrally administered with NA. However, naftopidil by itself had no effect on either parameter. These findings can contribute towards explaining the mechanism by which naftopidil ameliorates nocturnal polyuria.

Conflicts of interest

Motokai Saito reports grants from Asahi Kasei Pharma, during the conduct of the study.

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