Role of FMRFamide-Activated Brain Sodium Channel in Salt-Sensitive Hypertension

Masato Nishimura, Ken Ohtsuka, Hakuo Takahashi, Manabu Yoshimura

Abstract—FMRFamide, a cardioexcitatory neuropeptide, directly activates a newly cloned amiloride-sensitive sodium channel that is expressed specifically in the brain and blocked by benzamil hydrochloride. In the present study, we investigated the effects of short- and long-term intracerebroventricular infusion of FMRFamide on arterial pressure, sympathetic activity, vasopressin release, and brain renin-angiotensin system genes in rats and studied the role of FMRFamide-activated brain sodium channels in salt-sensitive hypertension. The intracerebroventricular preinjection of FMRFamide and subsequent intracerebroventricular infusion of 0.15 mol/L NaCl increased mean arterial pressure (FMRFamide: 30 nmol/kg +13±2.6 mm Hg, P<0.01; 100 nmol/kg +21±1.8 mm Hg, P<0.01), heart rate, abdominal sympathetic activity, and plasma vasopressin concentration compared with vehicle. The intracerebroventricular coplexion of either benzamil or CV-11974 abolished these increases. In rats administered a high-salt diet (8% NaCl), the continuous intracerebroventricular infusion of FMRFamide (50 and 200 nmol \cdot kg^{-1} \cdot d^{-1}) for 5 days increased mean arterial pressure, heart rate, urinary excretion of vasopressin and norepinephrine, and mRNAs of renin, angiotensin I–converting enzyme, and angiotensin II type 1 receptor in hypothalamus and brain stem compared with vehicle. These increases were abolished by intracerebroventricular coplexion of benzamil. In rats administered a low-salt diet (0.3% NaCl), however, increases in these variables were smaller than those in rats receiving a high-salt diet. Together, these findings suggest that brain FMRFamide-activated sodium channels may be involved in the mechanism of salt-sensitive hypertension through regulation of the brain renin-angiotensin system. (Hypertension. 2000;35:443-450.)

Key Words: hypertension, sodium-dependent \textbullet renin-angiotensin system \textbullet brain \textbullet nervous system, sympathetic

The peptide Phe-Met-Arg-Phe-NH₂ (FMRFamide) is an excitatory neuropeptide present in both invertebrate and vertebrate nervous systems. Although the systemic or intracerebral administration of FMRFamide increases arterial pressure and sympathetic nerve activity (SNA) in rats, the mechanism of these pressor and sympathoexcitatory actions of FMRFamide has not been precisely determined. Recently, it was reported that FMRFamide induces a fast excitatory depolarizing response due to the direct activation of an amiloride-sensitive sodium channel. This FMRFamide-activated sodium channel is blocked by amiloride and benzamil hydrochloride, an amiloride analog and specific inhibitor of amiloride-sensitive sodium channels, but not by ethylisopropylamiloride, an efficient Na⁺/H⁺ exchanger inhibitor, indicating that this channel is a pure sodium channel. In addition, this FMRFamide-activated sodium channel shares low sequence identity with previously cloned epithelial sodium channel subunits, and the gene of this channel is expressed specifically in the brain.

We previously reported that brain sodium channels that are blocked by benzamil hydrochloride may be involved in the central pressor mechanisms of salt-sensitive hypertensive models such as deoxycorticosterone acetate (DOCA)-salt hypertensive or stroke-prone spontaneously hypertensive rats, possibly through participation in the regulation of SNA or arginine vasopressin release. Furthermore, we recently showed that benzamil-blockable brain sodium channels likely play a role in the regulation of brain renin-angiotensin system genes. Amiloride-sensitive sodium channels reportedly play an important role not only in the transmembrane transport of sodium but also in sodium taste transduction as sodium receptors in lingual epithelial cells. These findings suggest that benzamil-blockable brain sodium channels may play a role in the pressor mechanism of salt-induced hypertension as a brain sodium receptor through participation in the regulation of the brain renin-angiotensin system.

In the present study, we investigated the effects of intracerebroventricular (ICV) preinjection of FMRFamide on changes in arterial pressure, heart rate, sympathetic activity, and vasopressin release with the subsequent ICV infusion of isotonic NaCl in rats. We also investigated the inhibitory effects of benzamil or angiotensin II type 1 (AT₁) receptor.
blocker on FMRFamide-induced responses. Furthermore, we investigated the effects of the continuous ICV infusion of FMRFamide on arterial pressure, heart rate, urinary excretion of vasopressin and norepinephrine, and gene expression of the brain renin-angiotensin system and confirmed the inhibitory effect of the ICV coinfusion of benzamil with FMRFamide on these variables.

Methods
Male Wistar rats aged 12 weeks (n=124, 250 to 270 g) purchased from Nihon-Dobutsu Co were housed in plastic cages at a constant temperature (22°C) with a 12-hour dark/light cycle. During the experiment, animals had free access to water and rat chow (Oriental Bio-Service Laboratory). The experimental procedure was approved by the Committee for Animal Research of Kyoto Prefectural University of Medicine.

Acute ICV Injection in Anesthetized Rats
Rats were anesthetized with urethane (120 mg/100 g IP; Nakarai Tesque) and mounted on a Kopf stereotaxic apparatus after the implantation of a femoral arterial catheter (PE-50; Clay Adams, Becton-Dickinson) filled with heparinized (50 U/mL) saline solution. Arterial pressure was continuously recorded by connecting the catheter to a small volume displacement pressure transducer (TP-200T; Nihon Kohden). Heart rate was automatically calculated based on the femoral arterial pulse wave pressure generating a tachometer (AT-601G; Nihon Kohden). The trachea was intubated with a cannula (PE-240; Clay Adams), which was connected to an artificial ventilator after skeletal muscle was paralyzed with an injection of decamethonium bromide (0.2 mg/100 g IV; Sigma Chemical Co) to the infusion cannula and implanted subcutaneously into the back of the body. The method has been described in detail elsewhere.3,8 aCSF (vehicle of FMRFamide, n=8), FMRFamide (100 nmol · kg⁻¹ · d⁻¹, n=6; 200 nmol · kg⁻¹ · d⁻¹, n=8), or FMRFamide plus benzamil hydrochloride (200 nmol · kg⁻¹ · d⁻¹, n=8) was infused intracerebroventricularly for 5 days. Metabolic studies were performed with the use of metabolic cages that were made in our laboratory. Twenty-four-hour urine samples were collected to measure diurnal urinary excretion of Na⁺, arginine vasopressin, and unconjugated free norepinephrine. At the end of the experiments, the rats were anesthetized through ether inhalation and killed through decapitation. Urinary Na⁺ concentration was measured with an automatic analyzer (Ektachem 700 analyzer; Eastman Kodak), and urinary concentrations of free norepinephrine and arginine vasopressin were measured with HPLC with electrochemical detection or radioimmunoassay as described previously.13 In a preliminary study, the continuous infusion of either FMRFamide (200 nmol · kg⁻¹ · d⁻¹) or aCSF into the right jugular vein for 5 days with use of the osmotic minipumps in rats treated with either the high- or low-salt regimen showed that compared with aCSF infusion, the continuous intravenous infusion of FMRFamide for 5 days did not affect mean arterial pressure (changes from baseline: high salt: aCSF +2±1 mm Hg, n=5; FMRFamide +1±2 mm Hg, n=5; low salt: aCSF +2±2 mm Hg, n=5; FMRFamide +2±1 mm Hg, n=5), heart rate (changes from baseline: high salt: aCSF -6±2 bpm, n=5; FMRFamide -4±2 bpm, n=5; low salt: aCSF -6±3 bpm, n=5; FMRFamide -3±2 bpm, n=5), or urinary excretion of vasopressin (changes from baseline: high salt: aCSF -1.2±1.4 pg · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -1.5±1.2 pg · g body weight⁻¹ · d⁻¹, n=5; low salt: aCSF -1.1±0.9 pg · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -1.3±1.5 pg · g body weight⁻¹ · d⁻¹, n=5) or norepinephrine (changes from baseline: high salt: aCSF -0.9±0.08 ng · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -0.8±0.08 ng · g body weight⁻¹ · d⁻¹, n=5; low salt: aCSF -1.0±0.12 ng · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -0.8±0.09 ng · g body weight⁻¹ · d⁻¹, n=5). The preliminary findings indicate that the effect of any possible leakage of the centrally administered drug into the peripheral blood is negligible in this long-term ICV infusion model.

Recording of Abdominal SNA
The abdominal plexus was exposed through transverse incision of the abdominal wall, and the abdominal sympathetic nerve bundle emerging from the celiac ganglion was placed over a bipolar electrode (uninsulated tips 1 mm apart). Spike potentials, which were amplified (Biophysiobaizer; NEC-Sanei Instrument Co Ltd), were monitored on a storage oscilloscope (Nihon Kohden) and continuously recorded together with arterial pressure on a magnetic tape recorder (TEAC Corp). Tapes were later played back into an amplitude analyzer to delete any background noise. Impulses were then fed to a spike counter (Dia Medical System Co Ltd), and the output, which was digitalized, was printed as a histogram while it was simultaneously recorded onto an inkless rectigraph. Integrated nerve activity was expressed as the percent change from baseline, obtained through counting of the number of spikes for 5 seconds. These percent changes were compared between groups.

Continuous ICV Infusion
Sixty-four rats were randomly divided into high- and low-salt groups (n=32 each). The high-salt group animals received a diet containing 8% NaCl and 1% NaCl solution as drinking water, and the low-salt group animals received a diet containing 0.3% NaCl and distilled water for 4 weeks before and during the experiment. Rats were anesthetized with sodium pentobarbital (50 mg/kg IP) 5 days before the start of the long-term ICV infusion and an arterial catheter (PE-50) filled with heparinized saline (50 U/mL) was inserted into the descending aorta through the right femoral artery. The other end of the catheter was pulled through a cut in the skin on the back of the neck at the level of the cervical vertebrae. Arterial pressure was recorded at the morning (8 to 11 AM) for 10 minutes once a day starting 3 days after implantation of the arterial catheter through the connection of the catheter tip to a small volume displacement pressure transducer as described earlier. After a 2-day control period to measure baseline values for arterial pressure, heart rate, and urinary excretion of vasopressin and norepinephrine, rats were placed on a stereotaxic frame while under anesthesia with sodium pentobarbital (50 mg/kg IP). An osmotic minipump (Alzet, model 2001; Alza Corp) filled with aCSF (FMRFamide vehicle), FMRFamide, or FMRFamide plus benzamil hydrochloride was connected to the infusion cannula and implanted subcutaneously into the back of the body. The method has been described in detail elsewhere.3,8 aCSF (FMRFamide vehicle, n=8), FMRFamide (50 nmol · kg⁻¹ · d⁻¹, n=8; 200 nmol · kg⁻¹ · d⁻¹, n=8), or FMRFamide plus benzamil hydrochloride (200 nmol · kg⁻¹ · d⁻¹, n=8) was infused intracerebroventricularly for 5 days. Metabolic studies were performed with the use of metabolic cages that were made in our laboratory. Twenty-four-hour urine samples were collected to measure diurnal urinary excretion of Na⁺, arginine vasopressin, and unconjugated free norepinephrine. At the end of the experiments, the rats were anesthetized through ether inhalation and killed through decapitation. Urinary Na⁺ concentration was measured with an automatic analyzer (Ektachem 700 analyzer; Eastman Kodak), and urinary concentrations of free norepinephrine and arginine vasopressin were measured with HPLC with electrochemical detection or radioimmunoassay as described previously.13 In a preliminary study, the continuous infusion of either FMRFamide (200 nmol · kg⁻¹ · d⁻¹) or aCSF into the right jugular vein for 5 days with use of the osmotic minipumps in rats treated with either the high- or low-salt regimen showed that compared with aCSF infusion, the continuous intravenous infusion of FMRFamide for 5 days did not affect mean arterial pressure (changes from baseline: high salt: aCSF +2±1 mm Hg, n=5; FMRFamide +1±2 mm Hg, n=5; low salt: aCSF -2±2 mm Hg, n=5; FMRFamide -2±1 mm Hg, n=5), heart rate (changes from baseline: high salt: aCSF -6±2 bpm, n=5; FMRFamide -4±2 bpm, n=5; low salt: aCSF -6±3 bpm, n=5; FMRFamide -3±2 bpm, n=5), or urinary excretion of vasopressin (changes from baseline: high salt: aCSF -1.2±1.4 pg · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -1.5±1.2 pg · g body weight⁻¹ · d⁻¹, n=5; low salt: aCSF -1.1±0.9 pg · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -1.3±1.5 pg · g body weight⁻¹ · d⁻¹, n=5) or norepinephrine (changes from baseline: high salt: aCSF -0.9±0.08 ng · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -0.8±0.08 ng · g body weight⁻¹ · d⁻¹, n=5; low salt: aCSF -1.0±0.12 ng · g body weight⁻¹ · d⁻¹, n=5; FMRFamide -0.8±0.09 ng · g body weight⁻¹ · d⁻¹, n=5). The preliminary findings indicate that the effect of any possible leakage of the centrally administered drug into the peripheral blood is negligible in this long-term ICV infusion model.
Isolation and Analysis of RNA
Immediately after decapitation, the brain (hypothalamus and lower brain stem) was removed, frozen in dry ice, and stored at −80°C until extraction. Total RNA was isolated according to the guanidine thiocyanate method as described previously. Quantitative analysis of the expression levels of renin, angiotensin I–converting enzyme (ACE), angiotensin II AT1 (AT1a plus AT1b) receptor, and angiotensinogen mRNAs was performed with a competitive PCR method as previously reported. Sample RNA (1 μg of total RNA) mixed with known amounts of the deletion-mutated cRNA underwent RT with the use of random primers. The amount of competitor cRNA used, the primer sequences, and the size of PCR product in each gene are previously reported. Sample RNA (1 μg of total RNA) mixed with known amounts of the deletion-mutated cRNA underwent RT with the use of random primers. The amount of competitor cRNA used, the primer sequences, and the size of PCR product in each gene are described in Table 1. The reaction profile included an initial denaturing step at 95°C for 1 minute and 40 cycles at 95°C for 30 seconds; 62°C (renin), 58°C (angiotensinogen, ACE), or 55°C (AT1 receptor) for 30 seconds; and 72°C for 1 minute. Because the mutated cRNA for ACE has a 4-bp insertion at the AvrII site, the PCR product from the mutated cRNA lacks this AvrII site. The PCR product from native ACE mRNA should liberate 195- and 122-bp fragments through AvrII digestion. The PCR product from the mutated cRNA does not liberate these fragments (Takara Shuzo Co Ltd) digestion. The RT-PCR methods are described in detail elsewhere.

Statistical Analysis
Data are expressed as mean±SEM. Differences between experimental and control groups were evaluated with one-way ANOVA, followed by the application of Duncan’s new multiple range test. A level of P<0.05 was accepted as statistically significant.

Results
Effect of ICV Preinjection of FMRFamide and Subsequent Infusion of Isotonic NaCl on Changes in Mean Arterial Pressure, Heart Rate, Sympathetic Activity, and Plasma Vasopressin Concentration in Anesthetized Rats
The ICV preinjection of FMRFamide increased mean arterial pressure and heart rate transiently 1 to 5 minutes after injection (peak change in mean arterial pressure: 30 nmol/kg FMRFamide +15±3 mm Hg, n=8; 100 nmol/kg FMRFamide +22±4 mm Hg, n=8; peak change in heart rate: 30 nmol/kg FMRFamide +25±8 bpm, n=8; 100 nmol/kg FMRFamide +34±10 bpm, n=8) but returned to baseline levels 10 minutes after the injection. Baseline levels of mean arterial pressure, heart rate, or abdominal SNA did not change between before and 15 minutes after the ICV preinjection (at the start of the ICV infusion of isotonic NaCl) with FMRFamide or FMRFamide plus benzamil or CV-11974 preinjection (Table 2).

<table>
<thead>
<tr>
<th>Target</th>
<th>Competitor Amount, molecules/μg</th>
<th>PCR Product Size</th>
<th>Primer Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renin</td>
<td>4.1×10⁴</td>
<td>372 bp (native)</td>
<td>5'-CTGGGAGGCGAGTGACCTCCACACTACCGAG-3'</td>
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<tr>
<td>ACE</td>
<td>5.5×10⁴</td>
<td>317 bp (native)*</td>
<td>5'-CCTGATCAACCAGAAGTTGCAGAG-3'</td>
</tr>
<tr>
<td>AGT</td>
<td>4.0×10⁴</td>
<td>810 bp (native)</td>
<td>5'-GACGGGATACATCACCCTTGCAGTCCTC-3'</td>
</tr>
<tr>
<td>AT1 receptor</td>
<td>2.5×10⁴</td>
<td>607 bp (native)</td>
<td>5'-GGAACAGTTGGTGGTGTG-3'</td>
</tr>
</tbody>
</table>

AGT indicates angiotensinogen. *Sensitive to AvrII digestion. †Resistant to AvrII digestion.

TABLE 2. Mean Arterial Pressure and Heart Rate Before and 15 min After ICV Preinjection of Either Vehicle, FMRFamide, FMRFamide Plus Benzamil, or FMRFamide Plus CV-11974 and Changes in Abdominal Sympathetic Nerve Activity Between Before and After the ICV Preinjections in Anesthetized Rats

<table>
<thead>
<tr>
<th>Target</th>
<th>Mean Arterial Pressure, mm Hg</th>
<th>Heart Rate, bpm</th>
<th>Δ Abdominal Sympathetic Activity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before ICV Preinjection</td>
<td>After ICV Preinjection</td>
<td>Before ICV Preinjection</td>
</tr>
<tr>
<td>Vehicle (n=8)</td>
<td>94±3</td>
<td>92±3</td>
<td>383±7</td>
</tr>
<tr>
<td>FMRFamide, 30 nmol/kg (n=8)</td>
<td>94±3</td>
<td>94±3</td>
<td>389±6</td>
</tr>
<tr>
<td>FMRFamide, 100 nmol/kg (n=8)</td>
<td>92±3</td>
<td>94±4</td>
<td>384±5</td>
</tr>
<tr>
<td>FMRFamide, 100 nmol/kg, + benzamil, 10 nmol/kg (n=8)</td>
<td>96±4</td>
<td>97±4</td>
<td>384±8</td>
</tr>
<tr>
<td>FMRFamide, 100 nmol/kg, + CV-11974, 50 μg/kg (n=8)</td>
<td>92±3</td>
<td>94±3</td>
<td>389±8</td>
</tr>
</tbody>
</table>
The ICV infusion of 0.15 mol/L isotonic NaCl caused no significant change in mean arterial pressure, heart rate, or abdominal sympathetic activity after ICV preadministration of the vehicle of FMRFamide. In contrast, the ICV preinjection of FMRFamide caused a dose-dependent increase in mean arterial pressure, heart rate, abdominal sympathetic activity, and plasma concentration of vasopressin on the subsequent ICV infusion of isotonic NaCl solution compared with the ICV preinjection of the vehicle (Figure 1). The ICV copreinjection of either 10 nmol/kg benzamil or 50 μg/kg CV-11974 with 200 nmol/kg FMRFamide abolished the increases in mean arterial pressure, heart rate, sympathetic activity, and plasma vasopressin concentration induced by the ICV preinjection of FMRFamide alone (Figure 1).

B

D

Figure 1. Effects of ICV preinjection of vehicle (C, aCSF, n = 8), 30 nmol/kg FMRFamide (A, n = 8), 100 nmol/kg FMRFamide (B, n = 8), 100 nmol/kg FMRFamide and 10 nmol/kg benzamil hydrochloride (D, n = 8), or 100 nmol/kg FMRFamide and 50 μg/kg CV-11974 (E, n = 8) on changes in mean arterial pressure (ΔMAP, A), heart rate (ΔHR, B), and abdominal SNA (ΔSNA, C) from baseline levels induced during ICV infusion of 0.15 mol/L isotonic NaCl solution for 30 minutes in anesthetized rats. D, Effects of ICV preinjection of vehicle (V, n = 8), 30 nmol/kg FMRFamide (F-30, n = 8), 100 nmol/kg FMRFamide (F-100, n = 8), 100 nmol/kg FMRFamide and 10 nmol/kg benzamil hydrochloride (F-100 and B, n = 8), or 100 nmol/kg FMRFamide and 50 μg/kg CV-11974 (F-100 and CV, n = 8) on plasma concentration of arginine vasopressin (AVP) after ICV infusion of 0.15 mol/L isotonic NaCl solution for 30 minutes. *P < 0.05, **P < 0.01 vs vehicle.

††P < 0.01 vs 100 nmol/kg FMRFamide.

Effect of Continuous ICV Infusion of FMRFamide for 5 Days on Hypertensive Variables on High- and Low-Salt Intake

In the high-salt group that received a diet containing 8% NaCl and 1% NaCl drinking water, the ICV infusion of 50 nmol · kg⁻¹ · d⁻¹ FMRFamide increased mean arterial pressure from day 2, urinary excretion of vasopressin and norepinephrine from day 3, and heart rate on day 5 of the infusion compared with the vehicle infusion. The ICV infusion of 200 nmol · kg⁻¹ · d⁻¹ FMRFamide further increased mean arterial pressure and urinary excretion of vasopressin and norepinephrine. In contrast, the ICV coinfusion of 10 nmol · kg⁻¹ · d⁻¹ benzamil with 200 nmol · kg⁻¹ · d⁻¹ FMRFamide abolished the increases in mean arterial pressure, heart rate and urinary excretion of vasopressin and norepinephrine induced by the ICV infusion of 200 nmol · kg⁻¹ · d⁻¹ FMRFamide alone (Figure 2A). Urinary Na⁺ excretion did not differ between the groups with the ICV infusion of vehicle, FMRFamide, or FMRFamide plus benzamil (Table 3).

In the low-salt group, however, the ICV infusion of 50 nmol · kg⁻¹ · d⁻¹ FMRFamide did not affect mean arterial pressure, heart rate, or urinary excretion of vasopressin or norepinephrine (Figure 2B). The ICV infusion of 200 nmol · kg⁻¹ · d⁻¹ FMRFamide increased mean arterial pressure from day 3 and urinary excretion of vasopressin and norepinephrine from day 4 of infusion, but these increases in the low-salt group were lower than those in the high-salt group (mean arterial pressure: day 3, 96 ± 2 [n = 8] versus 115 ± 4 [n = 8] mm Hg, P < 0.01; day 4, 97 ± 3 [n = 8] versus 132 ± 5 [n = 8] mm Hg, P < 0.01; day 5, 99 ± 3 [n = 8] versus 132 ± 4 [n = 8] mm Hg, P < 0.01; urinary vasopressin: day 4, 19.9 ± 1.6 [n = 8] versus 39.6 ± 4.6 [n = 8] pg · kg⁻¹ · d⁻¹, P < 0.01; day 5, 18.7 ± 1.3 [n = 8] versus 36.5 ± 4.3 [n = 8] pg · kg⁻¹ · d⁻¹, P < 0.01; urinary norepinephrine: day 4, 2.1 ± 0.07 [n = 8] versus 2.7 ± 0.16 [n = 8] ng · kg⁻¹ · d⁻¹, P < 0.01; day 5, 2.3 ± 0.1 [n = 8] versus 3.1 ± 0.2 [n = 8] ng · kg⁻¹ · d⁻¹, P < 0.01). The increases in mean arterial pressure and urinary excretion of vasopressin and norepinephrine induced by the ICV infusion of 200 nmol · kg⁻¹ · d⁻¹ FMRFamide were inhibited by the ICV coinfusion of 10 nmol · kg⁻¹ · d⁻¹ benzamil (Figure 2B). Urinary Na⁺ excretion did not differ between the groups with the ICV infusion of vehicle, FMRFamide, or FMRFamide plus benzamil (Table 3).
Effect of Continuous ICV Infusion of FMRFamide on Renin-Angiotensin System mRNAs on High- and Low-Salt Intake

In the high-salt group, the ICV infusion of 50 nmol · kg\(^{-1}\) · d\(^{-1}\) FMRFamide increased renin mRNA in the hypothalamus compared with the vehicle infusion, and that of 200 nmol · kg\(^{-1}\) · d\(^{-1}\) FMRFamide increased ACE and AT\(_1\) receptor mRNAs as well as renin mRNA in the hypothalamus (Figure 3A). The ICV infusion of benzamil not only abolished these increases in renin, ACE, and AT\(_1\) receptor mRNAs in the low-salt group. The ICV infusion of 200 nmol FMRFamide increased renin mRNA, although this increase was lower than that in the high-salt group and did not affect ACE or AT\(_1\) receptor mRNAs. The ICV infusion of FMRFamide did not affect angiotensinogen mRNA in either group.

Discussion

In the present study, the ICV preinjection of FMRFamide and the subsequent ICV infusion of isotonic NaCl caused a significant increase in mean arterial pressure, heart rate, abdominal SNA, and plasma vasopressin concentration. In contrast, the ICV preinjection of vehicle of FMRFamide and the subsequent ICV infusion of isotonic NaCl elicited no change in these variables. The ICV copreinjection of either benzamil or CV-11974 with FMRFamide abolished these increases on the subsequent ICV infusion of isotonic NaCl. Because FMRFamide reportedly activates the amiloride-sensitive sodium channel, which is specifically present in the brain and is blocked by benzamil, these results indicate that the increases in these variables on the ICV preinjection of FMRFamide and the subsequent ICV infusion of isotonic NaCl may involve the FMRFamide-activated brain sodium channel, the brain renin-angiotensin system, or both.

Cerebrospinal fluid (CSF) is produced at a constant rate of 2 \(\mu\)L/min in anesthetized rats;\(^{15}\) Na\(^+\) is thought to be produced at \(\approx\) 292 nmol/min (8.76 \(\mu\)mol/30 min) because Na\(^+\) concentration in the CSF of male Wistar rats is reported to be 146 mmol/L.\(^{16}\) The ICV infusion of isotonic NaCl in this study should have added Na\(^+\) to CSF by 75 nmol/min (2.25 \(\mu\)mol/30 min); this amount of Na\(^+\) corresponds to \(\approx25\%\) of Na\(^+\) spontaneously produced in the CSF of rats. Na\(^+\) concentration in CSF, however, is reported not to be significantly altered during the ICV infusion of isotonic NaCl because transient increase in Na\(^+\) concentration is buffered by free water movement from extraventricular spaces down the osmotic gradient.\(^{16}\) Therefore, an increase in cerebrospinal Na\(^+\) concentration should have been small on the ICV infusion of isotonic NaCl in this study. In previous studies that showed pressor effect of intracerebral injection of FMRFamide, FMRFamide was administered into the brain together with small amount of Na\(^+\) because FMRFamide was dissolved by isotonic NaCl solution.\(^{5,17}\) Pressor response induced by the ICV injection of FMRFamide without the addition of Na\(^+\) is lower than that with the addition of isotonic NaCl in rats (unpublished data). Results in the acute experiment portion of the present study suggest that FMRFamide preinjected into the brain activated benzamil-blockable brain sodium channel and that this activated brain sodium channel perceived a small increase in cerebrospinal Na\(^+\) concentration.

200 nmol · kg\(^{-1}\) · d\(^{-1}\) FMRFamide affected neither ACE nor AT\(_1\) receptor mRNAs in the low-salt group. The ICV infusion of FMRFamide did not affect angiotensinogen mRNA in either group.

In the lower brain stem, changes in renin-angiotensin system mRNAs were closely similar to those in hypothalamus (data not shown). In the high-salt group, the ICV infusion of 50 or 200 nmol · kg\(^{-1}\) · d\(^{-1}\) FMRFamide increased renin, ACE, and AT\(_1\) receptor mRNAs. These increases were abolished by the ICV coinfusion of benzamil. In the low-salt group, the ICV infusion of 200 nmol FMRFamide increased renin mRNA, although this increase was lower than that in the high-salt group and did not affect ACE or AT\(_1\) receptor mRNAs. The ICV infusion of FMRFamide did not affect angiotensinogen mRNA in either group.
The blockade of increases in arterial pressure, heart rate, sympathetic activity, and vasopressin release on the ICV preinjection of FMRFamide and the subsequent ICV infusion of isotonic NaCl by the ICV copreinjection of CV-11974 indicates that these FMRFamide-induced responses involve the action of angiotensin II via brain AT1 receptor. It is well known that brain angiotensin II stimulates vasopressin release and sympathetic activity. Previous reports have shown that the pressor effect of hypertonic NaCl on the ICV infusion is blocked by angiotensin II receptor antagonists\(^{18,19}\) and that the presence of Na\(^+\) in angiotensin II solution is necessary to produce a pressor effect when angiotensin II is injected into the cerebral ventricles.\(^{20}\) In addition, we have found that the enhanced responses in arterial pressure, sympathetic activity, and vasopressin release induced by the ICV infusion of angiotensin II are abolished by the ICV preinjection of benzamil (unpublished data). Although the present study does not precisely elucidate the relationship between the sodium channel and renin-angiotensin system in the brain, these findings suggest that FMRFamide-activated sodium channel is involved in the possible role of the brain renin-angiotensin system in the pressor mechanism of salt-induced hypertension.

The continuous ICV infusion of FMRFamide increased mean arterial pressure, heart rate, and urinary excretion of vasopressin and norepinephrine in rats administered a high-salt diet. In contrast, increases in these variables were lower in rats treated with a low-salt diet. These findings suggest that the continuous ICV infusion of FMRFamide elicits a salt-sensitive hypertension in rats. The hypertension induced by the continuous ICV infusion of FMRFamide is not likely to be due to increase in sodium retention, because urinary excretion of Na\(^+\) did not differ among rats with long-term ICV infusions of vehicle, FMRFamide, or FMRFamide plus benzamil, although sodium retention cannot be evaluated solely on the basis of urinary Na\(^+\) excretion. Because increases in both arterial pressure and urinary excretion of vasopressin and norepinephrine were abolished by the ICV coinfusion of benzamil with FMRFamide, the genesis of this salt-sensitive hypertension is expected to involve benzamil-blockable FMRFamide-activated sodium channel in the brain. This activated brain sodium channel may have perceived a small increase in the cerebrospinal Na\(^+\) concentration induced by the long-term administration of a high-salt diet and elicited the central pressor mechanisms such as enhanced sympathetic activity and vasopressin release.

In our recent study, we showed that upregulation of gene expression of the tissue renin-angiotensin system, including renin, ACE, and AT1 receptor, is greater in the brain than in the kidney in DOCA-salt hypertensive rats and that the ICV infusion of benzamil attenuated hypertension and abolished increases in urinary excretion of vasopressin and norepinephrine and in renin, ACE, and AT1 receptor mRNAs in the brain.\(^{8}\) In the present study, the continuous ICV infusion of FMRFamide elicited upregulation of renin, ACE, and AT1 receptor mRNAs in the brain, together with increases in arterial pressure and urinary excretion of vasopressin and norepinephrine. These changes were all abolished by the ICV coinfusion of benzamil. Thus, these phenomena are closely similar to those found in DOCA-salt hypertensive rats. In a previous study, the continuous ICV infusion of angiotensin II caused salt-sensitive hypertension in rats.\(^{21}\) Together, these findings indicate that the genesis of hypertension in salt-induced hypertensive models such as DOCA-salt hypertension through the upregulation of the brain renin-angiotensin system genes involves not epithelial sodium channels, as previously reported,\(^{22}\) but rather newly cloned amiloride-sensitive sodium channels in the brain that are activated by FMRFamide.\(^{6}\) FMRFamide-activated brain sodium channel may participate in the upregulation of the brain renin-angiotensin system genes.

### TABLE 3. Urinary Na\(^+\) Excretion During Continuous ICV Infusions of Either Vehicle, FMRFamide, or FMRFamide Plus Benzamil

<table>
<thead>
<tr>
<th></th>
<th>Vehicle (n=8)</th>
<th>FMRFamide, 50 nmol · kg(^{-1}) · d(^{-1}) (n=8)</th>
<th>FMRFamide, 200 nmol · kg(^{-1}) · d(^{-1}) (n=8)</th>
<th>FMRFamide, 200 nmol · kg(^{-1}) · d(^{-1}) + benzamil, 10 nmol · kg(^{-1}) · d(^{-1}) (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High salt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>66±5</td>
<td>67±7</td>
<td>65±6</td>
<td>68±5</td>
</tr>
<tr>
<td>Day 1</td>
<td>67±6</td>
<td>65±5</td>
<td>65±7</td>
<td>64±7</td>
</tr>
<tr>
<td>Day 3</td>
<td>64±7</td>
<td>68±8</td>
<td>64±8</td>
<td>65±7</td>
</tr>
<tr>
<td>Day 5</td>
<td>63±6</td>
<td>67±7</td>
<td>65±6</td>
<td>63±6</td>
</tr>
<tr>
<td><strong>Low salt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>17±4</td>
<td>16±3</td>
<td>17±3</td>
<td>17±4</td>
</tr>
<tr>
<td>Day 1</td>
<td>17±3</td>
<td>17±4</td>
<td>19±5</td>
<td>15±3</td>
</tr>
<tr>
<td>Day 3</td>
<td>16±3</td>
<td>15±4</td>
<td>19±6</td>
<td>19±5</td>
</tr>
<tr>
<td>Day 5</td>
<td>18±4</td>
<td>17±5</td>
<td>20±6</td>
<td>17±5</td>
</tr>
</tbody>
</table>

Urinary Na\(^+\) excretion was measured on the 2nd day of the control period and on the 1st, 3rd, and 5th days of the experimental period.
Benzamil-blockable brain sodium channel is thought to be activated in salt-sensitive hypertension models such as DOCA-salt hypertensive rats or stroke-prone spontaneously hypertensive rats. It is not clear from this study, however, whether high sodium itself recruits an FMRFamide-activated sodium channel in the brain. Although arterial pressure did not differ between vehicle-treated rats administered high- or low-salt diets, the ICV coinfusion of benzamil with FMRFamide decreased the mRNAs of renin, ACE, and AT1 receptor in the brain compared with the ICV infusion of vehicle in high-salt diet–treated rats, but not in low-salt diet–treated rats. Considering that benzamil-blockable FMRFamide-activated sodium channel may upregulate gene expression of renin, ACE, and AT1 receptor in the brain, these findings suggest that FMRFamide-activated sodium channel is also activated to some extent by high-salt administration. In addition, high-salt administration may be necessary for FMRFamide-activated brain sodium channel to elicit central pressor responses. Further study is needed to clarify whether FMRFamide content is increased in the brain in rats administered a high-salt diet or in salt-sensitive hypertensive rats.

Immunofluorescence study revealed the existence of an extensive system of FMRFamide-positive immunofluorescent nerve cell bodies and nerve fibers around the brain ventricle in the preoptic region of the frog brain. Moreover, numerous immunoreactive fibers were identified around the lateral ventricles in the forebrain and in the thalamus, hypothalamus, optic tectum, and medulla in frog brain. In rat brain, FMRFamide-like immunoreactive material is located in nerve cells and terminals, particularly in the nucleus accumbens, medial thalamus, hypothalamus, brain stem, and pituitary gland. On the other hand, the location of FMRFamide-activated sodium channels in the brain has not been precisely determined, notwithstanding that the gene of this channel is expressed particularly in the brain. Further study is needed to clarify the relationship between the physiological actions of this sodium channel and its distribution in the brain.

The cause of salt-sensitive hypertension has been largely related to the sodium excretion capacity of the kidney. In salt-sensitive hypertensive models, excessive salt intake and lowered sodium excretion elicit sodium retention in body fluid, which when continued stimulates centrally mediated pressor responses such as an increase in sympathetic activity or vasopressin release. However, the mechanism to explain how these centrally mediated pressor responses are elicited in salt-sensitive hypertensive models has not been precisely determined. FMRFamide-activated brain sodium channel is not likely to enhance sodium retention but may be involved in the centrally mediated pressor mechanisms of salt-sensitive hypertension, mainly through participation in the regulation of the brain renin-angiotensin system. We suggest that the FMRFamide-activated brain sodium channel is an important factor in explaining the mechanism of salt-sensitive hypertension.

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References


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