Brain Amiloride-Sensitive Phe-Met-Arg-Phe-NH₂–Gated Na⁺ Channels and Na⁺-Induced Sympathoexcitation and Hypertension

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Abstract—Dietary and cerebrospinal fluid (CSF) Na⁺ may act through brain amiloride-sensitive, Phe-Met-Arg-Phe-NH₂ (FMRFamide)-gated Na⁺ channels (FaNaChs) to cause sympathoexcitation and hypertension. We hypothesized that FaNaChs cause sympathoexcitation via the activation of brain “ouabain” and the brain renin-angiotensin system. In conscious Wistar rats, intracerebroventricular (ICV) infusion of Na⁺-rich (0.3 mol/L) artificial CSF (aCSF) and ICV injection of angiotensin II or ouabain increase renal sympathetic nerve activity (RSNA), blood pressure (BP), and heart rate (HR). ICV benzamil, an amiloride analogue, did not affect baseline values and blocked the responses to ICV infusions of Na⁺-rich aCSF but not ICV angiotensin II or ouabain. ICV FMRFamide also increased RSNA, BP, and HR. Blocking brain “ouabain” with ICV antibody Fab fragments abolished the responses to both ICV FMRFamide and Na⁺-rich aCSF. In conscious spontaneously hypertensive rats (SHR) on a high salt intake for 6 weeks, prolonged ICV but not intravenous infusion of benzamil at 10 to 20 μg/h significantly decreased RSNA, BP, and HR in a dose-related manner. The extent of these responses was significantly smaller in SHR on regular salt intake. These findings suggest that benzamil-blockable brain FaNaChs represent the major mechanism through which a small increase in CSF Na⁺ by ICV Na⁺-rich aCSF in Wistar rats or high salt intake in SHR initiates sympathoexcitation and hypertension. Enhanced Na⁺ entry through FaNaChs appears to activate brain “ouabain” and the brain renin-angiotensin system and, thereby, increases the sympathetic outflow. Brain FaNaChs appear to contribute to the worsening of hypertension in SHR on a high salt diet and, to a small extent, to the maintenance of hypertension in SHR on a regular salt diet. (Hypertension. 2002;39[part 2]:557-561.)

Key Words: brain ■ renin-angiotensin system ■ sympathetic nervous system ■ ouabain ■ sodium

Recent studies have indicated that brain ouabain-like substances (“ouabain”) and the brain renin-angiotensin system (RAS) contribute to sympathoexcitation and hypertension in normotensive/hypertensive rats with central sodium loading1,2 or salt-sensitive rats with a high salt intake.3-5 These studies did not establish how Na⁺ may activate central mechanisms, leading to increases in brain “ouabain.” Studies by Gomez-Sanchez and Gomez-Sanchez6 have shown that chronic central infusion of benzamil prevents the development of hypertension in Dahl salt-sensitive (Dahl S) rats on a high salt intake. Benzamil is an analogue of amiloride and blocks brain Phe-Met-Arg-Phe-NH₂ (FMRFamide)-gated Na⁺ channels (FaNaChs).7,8 These findings6 would suggest that brain amiloride-sensitive FaNaChs mediate Na⁺-induced sympathoexcitation and hypertension. In rats, FMRFamide–like immunoreactive material has been demonstrated in nerve cells and terminals, especially in the hypothalamus,9 but the existence of the FaNaChs, per se, has not yet been established in rats. In normotensive rats, intracerebroventricular (ICV) injection of FMRFamide increases blood pressure (BP), and this pressor effect can be prevented by ganglionic blockade, suggesting a sympathoexcitatory effect of central FMRF-amide.10 In Wistar rats, ICV injection of FMRFamide enhances excitatory responses of BP, heart rate (HR), and renal sympathetic nerve activity (RSNA) to ICV hypertonic saline, and this enhancement can be prevented by ICV benzamil or losartan, an angiotensin II (Ang II) type 1 (AT₁) blocker.11 Wistar rats on a high salt intake do not develop hypertension,12 but high salt plus chronic ICV infusion of FMRFamide increases BP and HR and is significantly associated with increases in hypothalamic renin, ACE, and AT₁ receptor mRNA.11 These responses are all prevented by ICV benzamil.11 It appears that the central Na⁺ concentration that is increased by an acute ICV infusion of hypertonic saline or a high salt intake activates the brain RAS through brain FaNaChs, thereby leading to sympathoexcitation and hypertension. We hypothesized that cerebrospinal fluid (CSF) Na⁺ stimulates brain “ouabain” through brain FaNaChs, leading to activation of the brain RAS and, thereby, sympathoexcitation and hypertension.

Hence, in the present study, we tested (1) whether, in Wistar rats, central sodium loading and activation of FaNaChs by ICV FMRFamide share a common pathway (ie, activation of brain “ouabain,” leading to sympathoexcitation
and hypertension), and (2) whether, in spontaneously hypertensive rats (SHR), prolonged ICV infusion of benzamil to block brain FaNaChs results in sympathoinhibition and lowering of BP and whether these occur to a greater degree in SHR on a high salt intake versus SHR on a regular salt intake.

Methods
Animal and Experimental Procedures
Six-week-old male Wistar rats (Charles River Laboratories, Montreal, Canada) and 3.5- to 4-week-old male SHR (Taconic Farms, Germantown, NY) were housed on a 12-hour light/dark cycle and were allowed a 5-day acclimatization on normal rat chow and tap water. SHR were placed on a regular salt (101 μmol Na+/g) or a high salt (1370 μmol Na+/g) diet from 4 to 10 weeks of age, and by 10 weeks, they had body weights of 250 ± 7 g versus 232 ± 8 g (P < 0.05). All procedures in the present study were carried out according to the guidelines of the University of Ottawa Animal Care Committee for the use and care of laboratory animals.

After the 5-day acclimatization in Wistar rats or at least 1 week before the end of the dietary period in SHR, a stainless-steel guide cannula was implanted with the rats under halothane anesthesia, and the cannula was fixed to the skull for injection or infusion into the right lateral cerebroventricle. Early in the morning ~1 week after brain surgery, with the rats under halothane anesthesia, catheters were inserted into the right femoral artery and vein. A pair of silver electrodes (A-M System, Inc) was placed around and fixed to the left renal nerve with silicone rubber (Siligel 604, Wacker) for the measurement of RSNA, as described previously. At least 4 hours after the rats had recovered from the anesthesia, the intrarterial catheter was connected to a pressure transducer, and BP and HR were recorded through a polygraph (model 7E, Grass Instrument Co). The electrodes were linked to a Grass P511 bandpass amplifier, and the amplified RSNA signals were channeled to a rectifying voltage integrator (model 7P10, Grass) and recorded through the polygraph. The RSNA signals (in millivolts) together with BP and HR were also fed into an online computer equipped with a Grass data acquisition and analysis program (Polyview 2.0). After a 30-minute rest, baseline mean arterial BP (MAP), HR, and RSNA were recorded in resting unrestrained animals.

Studies in Wistar Rats
Experiment I
At a 10-minute interval, artificial CSF (aCSF) and Na+ (0.3 mol/L)-rich aCSF were infused intracerebroventricularly at 3.8 μL/min for 5 and 10 minutes, respectively, through a needle inserted into the ICV guide cannula. Twenty minutes after the responses had subsided, 2 doses (20 and 40 ng in 2 to 4 μL aCSF) of Ang II were injected intracerebroventricularly at a 5-minute interval. The rats rested for 30 minutes, and then benzamil (3.5 μg/kg in 2 to 3 μL vehicle) or benzamil vehicle (15% propylene glycol in aCSF) was injected intracerebroventricularly. Ten minutes later, ICV administration of Na+-rich aCSF and of Ang II was repeated in a random order. Subsequently, after a 20-minute rest, ouabain (0.5 μg/2 μL) was injected intracerebroventricularly. Five minutes before each ICV administration, the vasopressin V1 receptor antagonist d(CH2)5-Tyr(Me)AVP (30 μg/kg in 0.1 mL saline, Sigma Chemical Co) was injected intravasally to exclude the responses to increased endogenous vasopressin by hypertonic saline and Ang II.

Experiment II
FMRFamide (100 nmol/kg in 2 to 4 μL aCSF) was first injected intracerebroventricularly. Twenty minutes after the responses had subsided, ouabain (0.5 μg) was injected intracerebroventricularly. The rats rested for 1 hour, and then antibody Fab fragments (132 μg/4 μL aCSF) were injected intracerebroventricularly. Five minutes later, ICV FMRFamide was repeated, and subsequently, Na+-rich aCSF was infused intracerebroventricularly, as described in experiment I. In another group of rats, the same protocol was repeated with γ-globulins (132 μg/4 μL) as a control for the Fab fragments. There were no differences in body weight (265 g), resting MAP (~110 mm Hg), and HR (~410 bpm) among the groups of Wistar rats in experiments I and II.

Studies in SHR
In SHR on a high salt or regular salt diet, after resting BP, HR, and RSNA had been recorded, benzamil was infused intracerebroventricularly or intravenously at 10 μg/h for 1 hour, followed by infusion at 20 μg/h for another 1 hour. In 1 group of SHR on a high salt diet, the vehicle for benzamil was infused intracerebroventricularly for 2 hours.

Data Analysis
A paired t test was used to compare the responses within the same group. One-way ANOVA was performed for comparisons between groups. When F ratios were significant, a Duncan multiple range test followed to locate the significant differences.

Results
Studies in Wistar Rats
Intravenous injection of an arginine vasopressin (AVP) antagonist, ICV infusion of aCSF, ICV injection of benzamil or its vehicle, or ICV injection of the Fab fragments or γ-globulins did not cause significant changes in resting hemodynamics and RSNA (data not shown).

Experiment I
Responses to Na+-rich aCSF
After pretreatment with an AVP antagonist, ICV infusion of Na+-rich aCSF increased MAP, RSNA, and HR (P < 0.05) within 2 minutes; plateau levels were reached in another 1 minute; and all parameters returned to resting levels within 2 minutes after the termination of the infusion. After ICV benzamil, ICV Na+-rich aCSF elicited only minor increases in MAP, RSNA, and HR. The extent of peak increases was significantly smaller than that observed before ICV benzamil (Figure 1A). In contrast, there were no significant differences between the responses to Na+-rich aCSF before and after ICV pretreatment with the vehicle for benzamil (Figure 1A).

Responses to ICV Ang II and Ouabain
After the AVP antagonist, ICV injection of Ang II (20 and 40 ng) increased MAP, RSNA, and HR in a dose-related manner. The extent of the peak responses was similar before and after the ICV injection of benzamil or its vehicle (Table). ICV injection of ouabain significantly increased MAP, RSNA and HR. The extent of the peak responses was similar with or without the ICV pretreatment with benzamil (Table).

Experiment II
Responses to ICV FMRFamide
ICV injection of 100 nmol FMRFamide caused parallel increases (P < 0.05) in MAP, RSNA, and HR. The responses were observed at 1 to 2 minutes, reached their peaks at 4 to 5 minutes, and had subsided by 10 to 12 minutes after the injection. Pretreatment with ICV Fab fragments abolished MAP, RSNA, and HR responses to FMRFamide, whereas pretreatment with ICV γ-globulins did not affect the responses (Figure 1B).
Results to Na⁺-rich aCSF

Compared with ICV γ-globulins, ICV pretreatment with Fab fragments abolished the excitatory MAP, RSNA, and HR responses to ICV infusion of Na⁺-rich aCSF (respective ICV γ-globulin versus ICV Fab values, 11±2 versus 3±1 mm Hg for MAP, 28±3% versus 2±1% for RSNA, and 30±3 versus 2±2 bpm for HR; *P<0.05 for all).

Studies in SHR

In SHR on a high salt diet, intravenous infusion of benzamil or ICV infusion of the vehicle for 2 hours did not change MAP, RSNA, and HR significantly. In contrast, after ICV infusion of benzamil (10 μg/30 μL per hour), MAP and RSNA started decreasing significantly at 15 to 20 minutes and reached plateau levels in another 5 to 10 minutes. Significant decreases in HR were observed at 50 minutes after the start of infusion. Increasing the rate of ICV benzamil infusion to 20 μg/30 μL per hour led to further decreases in MAP and RSNA but not in HR (Figure 2).

In SHR on a regular salt diet, significant decreases in MAP and RSNA were observed at 30 to 40 minutes after ICV benzamil at 10 μg/h, and decreases in HR were observed at 20 minutes after ICV benzamil at 20 μg/h. The extent of decreases in MAP and RSNA by ICV benzamil was significantly smaller in SHR on a regular salt diet versus SHR on a high salt diet (Figure 3). At the end of the 2-hour ICV benzamil infusion, MAP was similar in SHR on a regular salt diet versus SHR on a high salt diet (134±6 versus 135±5 mm Hg, respectively).

Discussion

In Wistar rats, ICV administration of Na⁺-rich aCSF, Ang II, ouabain, or FMRFamide caused similar increases in RSNA, BP, and HR. ICV pretreatment with benzamil inhibited the excitatory responses to ICV Na⁺-rich aCSF but not to ICV Ang II and ouabain. ICV pretreatment with the antibody Fab fragments to block brain "ouabain" abolished the excitatory responses to ICV FMRFamide as well as Na⁺-rich aCSF. In SHR, prolonged ICV but not intravenous infusion of benzamil decreased RSNA, BP, and HR in parallel. The decrease was 2- to 3-fold greater in SHR on a high salt intake versus SHR on a regular salt intake.

Chronic central Na⁺ loading in rats2,3 or high salt intake in salt-sensitive SHR or Dahl S rats4-5 elicits sympathetic hyperactivity and hypertension, associated with increases in CSF Na⁺ concentration,1,2,13,15 as well as increased brain hyperactivity and hypertension.
either benzamil or an AT1 blocker. Wistar rats are salt resistant and do not develop hypertension on a high salt intake. However, in Wistar rats on a high salt diet combined with chronic ICV infusion of FMRFamide, BP and HR significantly increase, in association with increases in hypothalamic renin, ACE, and AT1 mRNA. Concomitant infusion of benzamil prevents all these excitatory responses.

The present study extends the previous studies and demonstrates the irrelationship between brain FaNaChs and brain “ouabain” in mediating the sympathoexcitatory response to increased CSF Na+. First, similar to the previous studies, ICV FMRFamide increases BP, RSNA, and HR in parallel, and the extent of these responses is comparable to those elicited by ICV Na+-rich aCSF, Ang II, or ouabain.

Second, the blockade of brain “ouabain” abolishes excitatory BP, RSNA, and HR responses to both ICV injection of FMRFamide or ICV infusion of Na+-rich aCSF, suggesting that the responses to both FMRFamide and increased central Na+ are mediated by brain “ouabain.” Third, ICV pretreatment with benzamil blocks excitatory BP, RSNA, and HR responses to ICV Na+-rich aCSF but has no effect on the responses to ICV Ang II or ouabain. From the present findings, the previous finding that ICV benzamil blocks the effects of ICV FMRFamide, and the ineffectiveness of the ICV Fab fragments on the responses to ICV Ang II, we propose the concept that an increase in CSF Na+ through brain FaNaChs activates brain “ouabain” and that the latter exerts sympathoexcitatory and pressor effects through the brain RAS (see Figure 4 for proposed model).

In SHR on a regular salt diet, ICV infusion of benzamil at 10 to 20 μg/h for 2 hours caused mild, yet significant, decreases in BP, RSNA, and HR, suggesting that brain FaNaChs play some functional role in the maintenance of hypertension in SHR. In our previous studies, blockade of brain “ouabain” or of central AT1 receptors did not affect the resting BP of SHR on a regular salt diet.

“ouabain” content and activity of the brain RAS. These pressor and sympathoexcitatory effects of central Na+ can be prevented or reversed by blockade of either brain “ouabain” or the brain RAS. Central administration of hypertonic saline, ouabain, brain tissue extracts containing “ouabain,” or Ang II causes similar increases in BP, RSNA, and HR, which can all be attenuated by ICV pretreatment with an AT1 blocker. In contrast, ICV pretreatment with the Fab fragments blocks the responses only to ICV hypertonic saline or ouabain but not to Ang II. Therefore, we proposed that increased CSF Na+ increases brain “ouabain,” and the latter activates the brain RAS, leading to sympathoexcitation and, thereby, hypertension. Brain areas such as the ventral anteromedial third vehicle and median preoptic nucleus appear to play an important role in this relay. The mechanisms through which an increase in CSF Na+ activates brain “ouabain” have not yet been studied. The present study suggests that a small increase in CSF Na+ elicits these responses through brain amiloride-sensitive FaNaChs. In snail neurons, the neuropeptide FMRFamide induces a fast excitatory depolarizing response by direct activation of FaNaChs. FaNaChs have not yet been established in mammals, but similar amiloride-sensitive channels have recently been cloned from mammalian nervous tissue.

Functional studies are consistent with the presence of a mammalian counterpart to the snail FaNaChs. In rats, central administration of FMRFamide increases BP and HR and, in a dose-related manner, enhances sympathoexcitatory and pressor responses to ICV infusion of 0.15 mol/L NaCl, which is only just higher than the regular CSF Na+ (0.145 mol/L). These effects can be prevented by ICV pretreatment with either benzamil or an AT1 blocker. Wistar rats are salt 

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a regular salt diet through other pathways. Alternatively, the large size of the Fab fragments or the relatively low doses used may not effectively block all the sympathoexcitatory effects originating from relevant NaNaChs. The extent of the decreases in BP, RSNA, and HR in response to ICV infusion of benzamil was significantly greater in SHR on a high salt diet versus SHR on a regular salt diet, and at the end of the 2-hour benzamil infusion, MAP had become the same for SHR on a high salt diet versus SHR on a regular salt diet. These observations suggest that benzamil-blockable brain FaNaChs play a pivotal role in the development of salt-induced sympathetic hyperactivity and hypertension in SHR.

In Dahl S rats, high salt intake causes hypertension associated with mild but significant increases in CSF Na+ concentration.15 ICV infusion of benzamil at doses that have no effect when given peripherally also prevents the salt-induced hypertension in Dahl S rats. Therefore, it appears that brain benzamil-blockable FaNaChs may contribute significantly to salt-induced sympathoexcitation and hypertension in Dahl S rats and SHR. Larger increases in CSF Na+, higher levels of brain FMRFamidé, or increased entry of Na+ through brain FaNaChs may contribute to the salt-induced hypertension in salt-sensitive versus salt-resistant rat strains (Figure 4). The present findings suggest that in addition to mediating the responses to an acute increase in CSF Na+ in normotensive rats, brain FaNaChs play a pivotal role in the development of salt-induced sympathoexcitation and hypertension in SHR on a high salt diet.

Amiloride inhibits not only Na+ channels but also Na+-H+ and Na+-Ca2+ exchange channels. Benzamil inhibits Na+ channels more specifically than does amiloride: compared with amiloride, benzamil at concentrations 10 times lower are needed to inhibit Na+ channels, but concentrations at least 10 times higher are required to inhibit Na+-H+ exchange channels.21 Benzamil could also lower sympathetic activity by changing neuronal Ca2+ via inhibition of Na+-Ca2+ exchange channels. However, this seems unlikely in the present study. First, in vitro, the inhibitory concentration of benzamil for Na+ channels is in the range of 10 nmol/L, whereas the inhibitory concentration for Na+-Ca2+ exchange channels is in the range of 10 μmol/L.21 Second, in the present study, benzamil did not affect sympathoexcitatory and pressor responses to ouabain. The latter causes excitatory responses by increasing intracellular Na+ and, thereby, intracellular Ca2+ via Na+-Ca2+ exchange channels.

In summary, the present study suggests that Na+ entry through benzamil-blockable brain FaNaChs is the major mechanism through which a small increase in CSF Na+ induced by ICV infusion of Na+-rich aCSF in Wistar rats or a high salt intake in SHR leads to sympathoexcitation and hypertension. Na+ entry through brain FaNaChs appears to activate brain "ouabain," and the latter causes sympathoexcitation and hypertension through activation of the brain RAS. Brain FaNaChs may also contribute to a minor extent to the maintenance of hypertension in SHR on a regular salt diet.

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References

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