The Na$^{+}$/H$^{+}$ exchanger (NHE) is involved in the regulation of cerebral arteriolar tone in rats. It is known as the major contributor to the Na$^{+}$ influx pathway in vascular smooth muscle cells (VSMCs). Because activation of this transporter mediates Na$^{+}$ influx, both K$^{+}$ and Ca$^{2+}$ influxes can also be altered. An increase in Na$^{+}$ influx, membrane potential and Ca$^{2+}$ influxes may also contribute to ischemic injury. The Na$^{+}$/H$^{+}$ exchanger also contributes to ischemia and reperfusion injury in the coronary circulation. Nonetheless, there is limited information on the role of NHE in the regulation of cerebral arteriolar tone and its mechanisms in vitro.

Methods. The internal diameter of isolated pressurized intracerebral arterioles in rats was monitored with an aid of an intravital microscope. To examine the basal activity of NHE, two kinds of Na$^{+}$/H$^{+}$ exchange inhibitors (FR183998 and 5-N,N-hexamethylenejamiloride) were administered in the arterioles. Subsequently, the authors studied the effects of nitric oxide (NO) synthase inhibitor (FR183998), Na$^{+}$/K$^{+}$-adenosine triphosphatase (NKA) inhibitor (ouabain), and the Na$^{+}$/Ca$^{2+}$ exchanger inhibitor (SEAO400) on the vascular response induced by either of the Na$^{+}$/H$^{+}$ exchange inhibitors.

Conclusions. The Na$^{+}$/H$^{+}$ exchanger activity is related to the basal activity of NHE in cerebral arteries. Therefore, NHE inhibition may have a role in the regulation of cerebral arteriolar tone. Furthermore, the authors in the present study examined the role of NHE in the regulation of cerebral arteriolar tone and its related mechanisms in vitro.

Key Words: cerebral circulation, Na$^{+}$/H$^{+}$ exchanger, nitric oxide synthase, Na$^{+}$/K$^{+}$-adenosine triphosphatase, Na$^{+}$/Ca$^{2+}$ exchanger.
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cations connected to cardiac ischemia. Moreover, NHE4 may contribute to cerebral ischemia. Note that NHE inhibitor prevented the progression of cerebral ischemic damage and edema following MCA occlusion in rats.16 In a transient occlusion model in rat MCA, NHE inhibitor also improved endothelial dysfunction induced by ischemic reperfusion injury.2 Few studies have been undertaken on NHE in the regulation of cerebral vasomotor response, however.14 To our knowledge, there has been no in vitro study concerning NHE in the regulation of cerebral arteriolar tone.

Thus we initiated the present study to determine the following: basal activity of NHE in the regulation of cerebral arteriolar tone in rats; and contributions of endogenous NO, NKA, and the Na+/Ca2+ exchanger in the NHE-induced vascular response in rat microcirculation in vitro.

**Materials and Methods**

All experimental protocols were approved by the Animal Ethics Committee of Shanshu University School of Medicine.

**Isolated Vessel Preparation**

The procedure for vessel preparation has been previously reported.1 Briefly, male Sprague-Dawley rats (Japan SLIC, Hamamatsu, Japan) weighing a mean of 358.4 ± 12.3 g (± standard error of the mean) were anesthetized with an intraperitoneal injection of pentobarbital sodium (65 mg/kg) and were killed. The brain was removed and transferred to a dissection chamber filled with physiological salt solution (see later). A penetrating arteriole was isolated from the MCA and transferred to a 10-ml vessel chamber containing two glass micropipettes. The arteriole was cannulated using a micropipette and secured with a nylon suture (suture diameter ~10 µm; Fig. 1). All experiments were conducted without intraluminal flow. Transmural pressure (60 mm Hg) was applied and monitored continuously. The vessel chamber was mounted on the stage of an intravital microscope (Olympus BX51-33; Olympus Co., Tokyo, Japan). The maximal passive internal diameter of the arteriole was determined with the aid of a micromanipulator. Extraluminal application of nitric oxide (NO) exchange inhibitor, together with 3-(N,N-hexamethylen)amiloride (3 mmol/L) and 0.1 mmol/L L-NMMA, an NOS inhibitor, significantly reduced the vascular response in vasoconstriction, the inhibitory effect in NHE inhibitor–induced vasoconstriction, the vascular response in rat microcirculation in vitro.

**Vessel Diameter Measurements**

The internal diameter of vessels was obtained using an objective lens (10×), a photo-eyepiece lens (5×), and a monochrome charge-coupled device camera (KCB-270A; KOCOM Co., Ltd., Seoul, South Korea) and was displayed on a TV monitor (TM-150S; Nikon Victor, Iwai, Japan). The nature of this internal diameter in response to vasoactive agents was manually measured with a custom-made diameter-detection device, calibrated with a stage micrometer (Nikon Instech Co., Ltd., Kanagawa, Japan), and recorded on a video recorder (HR-3000; Nikon Victor) and a strip chart recorder (VP-6712A; Nikon National Instruments, Tokyo, Japan).

**Experimental Protocol**

To determine the role of NHE in the regulation of cerebral arteriole tone in basal conditions, two NHE inhibitors (5-(N,N-hexamethylene)amiloride and FR183998) were evaluated in the first series of experiments. Vascular response to 3-(N,N-hexamethylene)amiloride and FR183998 (10 mmol/L) was completely abolished in the control basal diameter. Ouabain significantly attenuated the vascular response in rat microcirculation in vitro.

**Results**

The maximal passive internal diameter was 75.9 ± 2.2 µm (22 vessels). All vessels developed spontaneous tone, constricting to a mean control basal diameter of 56 ± 1.8 µm. Both 5-(N,N-hexamethylene)amiloride and FR183998 constricted arterioles in a dose-dependent manner (Fig. 2 left and right). Extraluminal application of 5-(N,N-hexamethylene)amiloride (3 mmol/L) significantly reduced the control basal diameter of cerebral arterioles (four vessels; p < 0.001, Bonferroni multiple comparisons test; Fig. 3). Subsequent treatment with 0.1 mmol/L L-NMMA further vasoconstriction (four vessels; p < 0.001 compared with control vessels and p < 0.05 compared with vessels treated with 3 mmol/L 5-(N,N-hexamethylene)amiloride alone, Bonferroni multiple comparisons test). Ouabain (0.1 mmol/L) alone transiently constricted vessels (28.4 ± 6.7% change in internal diameter; five vessels), after which the internal vessel diameter returned to the control basal diameter. Ouabain significantly attenuated

![Fig. 1. Photomicrograph depicting a rat cerebral arteriole cannulated with a micropipette on both ends. Bar 50 µm.](image)
ed FR183998-induced constriction (five vessels; \( p = 0.011 \), paired Student t-test; Fig. 4).

Although SEA0400 (1 \( \mu \text{mol/L} \)) had no effect on the control basal diameter, it did inhibit the constriction caused by FR183998 (five vessels; \( p < 0.001 \), Bonferroni multiple comparisons test; Fig. 5). In the presence of SEA0400 (1 \( \mu \text{mol/L} \)), additional treatment with ouabain (0.1 \( \mu \text{mol/L} \)) also induced temporary constriction of vessels, but this constriction (7.5 ± 2.4% change of the internal diameter) was significantly attenuated. The combination of SEA0400 and ouabain totally abolished constriction caused by FR183998 (five vessels; \( p < 0.001 \) compared with control vessels and \( p < 0.001 \) compared with vessels treated with SEA0400, Bonferroni multiple comparisons test).

**Discussion**

In the present study, we characterized for the first time the basal activity of NHE in the regulation of cerebral arteriolar tone in vitro.

***Basal NHE Activity in Intracerebral Arterioles in Rats***

In rat intracerebral arterioles, there are many factors controlling the basal tone, such as NO, K+ channels, and Ca2+ channels. It is known that the inhibition of NKA by ouabain, a cardiac glycoside, increases [Na+]i and results in hyperpolarization. The transmembrane Na+/H+ exchanger and cerebral arteriolar tone are known to be inversely related to the intracellular pH, and changes in CO2 content may not be involved in NHE inhibitor–induced vasoconstriction (two vessels; data not shown). This result indicates that intracellular acidosis does not affect pHi. Nonetheless, the alteration of pH may be attributable to the difference in the regulatory mechanism of basal tone in territories of cerebral circulation. Note that there are regional differences in vascular responses between anterior and posterior circulation or between cerebral artery and arteriole according to data from previous studies.12 44

It has been reported that the activation of NHE causes vasocostriction in renal resistance vessels and iliac arteries in rats through an increase in [Na+]i.21 23 Our results are in opposition to these data because the basal activity of NHE dilates the intracerebral arterioles.

***Role of Endogenous NO in NHE-Induced Vascular Response***

Kitazono, et al.,19 reported that intracellular alkalinization produced by NHE may enhance NO production in the rat BA endothelium and thereby contribute to dilatory response. It is possible that the vasodilatory effect of NHE is not involved in NHE inhibitor–induced vasoconstriction. In preliminary experiments to test the relationship between intracellular acidosis and the vascular response in rat cerebral arterioles, it was also induced temporary constriction of vessels; thus, treatments with an NOS inhibitor following the administration of an NHE inhibitor caused no significant differences in vascular responses between anterior and posterior circulation or between cerebral artery and arteriole according to data from previous studies.12 44

The Na+/H+ exchanger activity of the artery in vivo is known to be responsible for regulating arteriolar tone through its vasodilatory effect. In contrast, Kitazono, et al.,18 reported that intracellular alkalinization produced by the NHE inhibitor, FR183998, decreases NO production in the rat cerebral arterioles. Our results are in opposition to these data because the basal activity of NHE dilates the intracerebral arterioles.
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BA endothelium and thereby contribute to dilatory responses of the artery in vivo. It may be possible that the basal activity of NHE stimulates production of NO. Nonetheless, our data indicate that endogenous NO production is not altered in the basal activity of NHE given that treatment with an NOS inhibitor following the administration of an NHE inhibitor caused further vasoconstriction.

In preliminary experiments to test the relationship between intracellular acidosis and the vascular response induced by the NHE inhibitor, 5 mmol/L methylammonium chloride, an intracellular alkalinizing agent, did not affect NHE inhibitor-induced vasoconstriction (two vessels; data not shown). This result indicates that intracellular acidosis is not involved in NHE inhibitor-induced vasoconstriction. It is possible that the vasodilatory effect of NHE in the basal state is caused by intracellular alkalinization and subsequent activation of NOS. The relationship between NHE and CO2 reactivity in the regulation of cerebral arteriolar tone can be explained by the fact that changes in CO2 content affect pH. Nonetheless, the alteration of pH caused by a change in CO2 content may not be involved in NHE inhibitor-induced vasoconstriction in rat cerebral arterioles.

Role of NKA and Na+/Ca2+ Exchanger in NHE-Induced Vascular Response

Note that NKA mediates the 2:3 exchange between [Na+] and [K+], and results in hyperpolarization. The transmembrane Na+ gradient by NKA stimulates NHE. It is well known that the inhibition of NKA by ouabain, a cardiac glycoside, increases [Na+], and has a direct vasoconstrictor effect on vascular smooth muscle. In the present study, ouabain partially attenuated NHE inhibitor–induced constriction; thus, basal activity of NHE may be regulated by NKA through [Na+].

In conditions that promote the elevation of [Na+], for example, enhanced Na+ entry or inhibition of NKA by cardiac glycosides, the Na+/Ca2+ exchanger may become activated. It has been reported that pretreatment with ouabain promotes Na+-dependent Ca2+ influx in cultured VSMCs. Therefore, the Na+/Ca2+ exchanger may play a role in the regulation of cytosolic-free [Ca2+]i, under conditions of [Na+]i loading through a sequence of inhibition of NKA activity, increased [Na+]i, and increased Ca2+ entry (or decreased Ca2+ extrusion) that is consistent with the direct vasoconstrictor effect observed with cardiac glycosides.

Analysis of data from previous studies revealed that the ouabain-promoted Na+-dependent Ca2+ influx in cultured VSMCs is not caused by voltage-dependent Ca2+ entry. The same conclusion has been drawn in regard to other types of smooth muscle. In our experiments, ouabain induced transient constriction, which was attenuated by SEA0400. This result indicates that transient constriction in rat cerebral arterioles caused by ouabain may be due to increased Ca2+ influx through the Na+/Ca2+ exchanger.

Vascular smooth-muscle cells display [Ca2+]i changes in response to the transmembrane Na+ gradient, which is consistent with the existence of Na+/Ca2+ exchangers. Thus, the activity of the Na+/Ca2+ exchanger is linked to the free [Na+]i gradient across the cell membrane, which is regulated by the activity of NHE and NKA. A change in [Na+]i may alter the activity of the Na+/Ca2+ exchanger and therefore change [Ca2+]i and vascular smooth-muscle tension. Analysis of data from previous studies indicates that Na+-dependent [Ca2+]i regulation in VSMCs via an Na+/Ca2+ exchanger operating as a Ca2+ efflux mode, possibly attributable to the inhibition of the Na+/Ca2+ exchanger working in the Ca2+ efflux mode. In a study of cultured VSMCs, the recovery of resting [Ca2+]i after stimulation by angiotensin II appears to be mediated by Ca2+ extrusion through the Na+/Ca2+ exchanger. In the present study, SEA0400, an Na+/Ca2+ exchange inhibitor, did not itself affect control diameter, thus indicating that the Na+/Ca2+ exchanger may be inactive in the basal condition. The finding that FR183998-induced vasoconstriction is attenuated by SEA0400 indi-
cates that the Na\(^{+/}/\)Ca\(^{2+/}\) exchanger works in a Ca\(^{2+/}\) influx mode in stimulated cerebral arterioles in the rats. Thus, NHE inhibitors may cause vasoconstriction through a sequence involving the activation of the Na\(^{+/}/\)Ca\(^{2+/}\) exchanger in the Ca\(^{2+/}\) influx mode and increased [Ca\(^{2+/}\)].

The NHE in Pathological Conditions

An increase in [Na\(^+\)] is well documented in ischemic myocardial cells and ischemic hearts.\(^{15,20,31}\) It has become clear that the increase in [Na\(^+\)] results from an imbalance between Na\(^{+/}\) influx and Na\(^{+/}\) extrusion in ischemic cells. In ischemic cells, intracellular acidosis activates NHE and causes increased Na\(^{+/}\) influx. On the other hand, the extrusion of Na\(^{+/}\) by the NKA decreases according to energy depletion.\(^{12,25,31}\) There are several diseases that may be associated with or induced by altered NHE activity, including hypertension, cardiac ischemia, and reperfusion injury.\(^{27,28}\)

An increased [Na\(^+\)] activates the Na\(^{+/}/\)Ca\(^{2+/}\) exchanger and results in an increase in free [Ca\(^{2+/}\)]. As expected, not only [Na\(^+\)] overload but also ischemic Ca\(^{2+/}\) overload results in a significant increase in [Ca\(^{2+/}\)].

It has been reported that inhibitors of NHE by FR183998 may have beneficial effects in reducing infarct volume and brain edema during cerebral ischemia in rats.\(^{17,22}\) Horikawa, et al. reported that the protective effect of Na\(^{+/}/\) H\(^{+/}\) exchanger on ischemic brain injury in rats may be at least partially mediated by the prevention of endothelial dysfunction. Nonetheless, the finding in this study that Na\(^{+/}/\) H\(^{+/}\) inhibitors induce significant vasoconstriction may raise a question about the effects of these drugs in cerebral microcirculation because significant vasoconstriction may lead to the reduction of cerebral blood flow and worsen ischemic injury. The role of NHE in cerebral ischemia may be different from its role in the normal physiological condition, however, and the exchange inhibitor may have a different effect on cerebral microcirculation.\(^{12,25}\)

Further studies are required to clarify the role of NHE and its inhibitors in cerebral microcirculation, especially in pathological conditions.

Conclusions

Analysis of data in the present study shows that NHE is active in the basal condition in rat intracerebral arterioles and regulates arteriolar tone through the dilatory response. Endogenous NO is not involved in basal NHE activity. The role of NHE in the regulation of arteriolar tone in rats correlates with the activities of the Na\(^{+/}/\)Ca\(^{2+/}\) exchanger and NKA.

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References

11. Horikawa N, Kuribayashi Y, Itoh N, et al: Na\(^{+/}/\)H\(^{+/}\) exchange inhibitors have cardioprotective effects.2,7,9,29,35,36 Recent studies have been reported that inhibition of NHE by FR183998 may have beneficial effects in reducing infarct volume and brain edema during cerebral ischemia in rats.17
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