Short Communication

Role of Na\(^+\)/Ca\(^{2+}\) Exchanger–Mediated Ca\(^{2+}\) Entry in Pressure-Induced Myogenic Constriction in Rat Posterior Cerebral Arteries

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Abstract. The involvement of Ca\(^{2+}\) entry via the Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) in myogenic constriction of rat posterior cerebral arteries was investigated. RT-PCR identified mRNA for NCX1, 2, and 3 in the arteries. Na\(^+\) removal increased \([\text{Ca}^{2+}]_{i}\), which was reduced by the NCX inhibitor SEA0400. SEA0400 inhibited the development, but not the steady-state, of pressure-induced myogenic constriction, whereas it decreased both the initial and sustained phases of \([\text{Ca}^{2+}]_{i}\) elevation. These results suggest that Ca\(^{2+}\) entry via NCX is involved in the development, but not the steady-state, of pressure-induced myogenic constriction.

Keywords: Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) 1, arteriograph, fura 2

Myogenic constriction contributes to autoregulation of blood flow to keep it nearly constant in small arteries and arterioles (1). Elevation of intraluminal pressure depolarizes vascular smooth muscle cells via membrane stretch, which in turn induces Ca\(^{2+}\) influx through voltage-dependent Ca\(^{2+}\) channels (VDCC) and vasocostriction. A large component of \([\text{Ca}^{2+}]_{i}\) elevation in the myogenic response is inhibited by VDCC blockers, and the remaining component is inhibited by blockers of non-selective cation channels (NSCC) (2). Since stretch-activated NSCC also pass Na\(^+\), the driving force for the Ca\(^{2+}\) entry mode of the Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) may be provided by the Na\(^+\) entry through NSCC. NCX transports Ca\(^{2+}\) either out of or into cells in exchange for 3 Na\(^+\). The direction of NCX-mediated Ca\(^{2+}\) movement depends on membrane potential and/or cytosolic Na\(^+\) or Ca\(^{2+}\) concentration (3). During adrenergic and purinergic stimulations, Na\(^+\) entry through NSCC has been shown to drive NCX-mediated Ca\(^{2+}\) entry in vascular smooth muscle (4, 5). In addition, the Ca\(^{2+}\) entry mode of NCX has been proposed to contribute to the increased vascular tone in salt-sensitive hypertensive rats (6). Recently, the Ca\(^{2+}\) entry mode of NCX has also been implicated to be involved in myogenic constriction of rat cremaster muscle arterioles (7). They showed that knockdown of NCX impaired both the development and steady-state of myogenic constriction (7). We have recently shown that at least two types of NSCC contribute to intraluminal pressure-induced myogenic constriction in rat posterior cerebral arteries (8). Moreover, the pressure stimulation induced a sustained \([\text{Ca}^{2+}]_{i}\) elevation even in the presence of a VDCC blocker (8). The present study was thus designed to elucidate the involvement of Ca\(^{2+}\) entry via NCX in myogenic constriction of cerebral arteries by using the specific NCX inhibitor SEA0400 (9, 10).

Male Wistar rats (200 – 300 g; SLC, Hamamatsu) were anesthetized with pentobarbital sodium (50 mg/kg, i.p.), and segments of the second branch of the posterior cerebral arteries with outer diameter between 100 – 200 μm were isolated. Changes in vessel diameter and \([\text{Ca}^{2+}]_{i}\) of the arterial segments loaded with fura 2-AM (4 μM; Dojindo, Kumamoto) were measured by arteriography as previously described (8). To extract total RNA from rat posterior cerebral arteries, we used the RNeasy Mini kit (Qiagen, Tokyo) according to the manufac-
turer’s instructions. In addition to the arteries, RNA was extracted from rat whole brain for a positive control. Samples were reverse-transcribed with specific primers and amplified by thermal cycling amplification.

RT-PCR identified the presence of mRNA for the three isoforms, NCX1, 2, and 3, in rat posterior cerebral arteries, although the expression of NCX2 mRNA was relatively low (Fig. 1A). Functional significance of the Ca\(^{2+}\) entry mode of NCX was also demonstrated: When the arterial segments were exposed to Na\(^+\)-free Tyrode’s solution, a transient [Ca\(^{2+}\)] elevation was induced (Fig. 1B). The [Ca\(^{2+}\)] elevation was rather low, which was 14.2 ± 0.6% (n = 4) of that induced by 60 mM K\(^+\). The NCX inhibitor SEA0400, which was synthesized by Taisho Pharmaceutical (Saitama), decreased the [Ca\(^{2+}\)] elevation in a concentration-dependent manner (Fig. 1B). SEA0400 has been shown to preferentially inhibit Ca\(^{2+}\) uptake by NCX1 compared with that by NCX2 and has a little inhibitory effect on Ca\(^{2+}\) uptake by NCX3 in CCL39 cells with a stable transfection of NCX isoforms (11). Taken together, NCX1 seems most probably to function in rat posterior cerebral arteries.

When the intraluminal pressure was raised from 10 to 60 mmHg, which is comparable to physiological intraluminal pressure in rat posterior cerebral arteries (12), the initial distention due to the pressure was followed by the development of sustained myogenic constriction (Fig. 2A), which was accompanied with [Ca\(^{2+}\)] elevation (Fig. 2B). In the presence of SEA0400 (1 μM), the development of the pressure-induced myogenic constriction was apparently slow in comparison with the control, but the amplitude of the steady-state constriction was comparable to it (Fig. 2A). SEA0400 significantly decreased the initial transient [Ca\(^{2+}\)] elevation and tended to decrease the steady-state one (Figs. 2: B and C). Similar inhibitory effects of SEA0400 (1 μM) on the development of myogenic constriction (Figs. 2: D and F) and the transient and sustained [Ca\(^{2+}\)] elevation (Figs. 2: E and F) were observed when the intraluminal pressure was raised from 10 to 100 mmHg. In addition, SEA0400 (1 μM) significantly decreased the [Ca\(^{2+}\)] (219.2 ± 5.4 and 202.4 ± 4.4 μM before and after the application of SEA0400, respectively; n = 4, P < 0.01), but had no effect on the constriction (85.9 ± 0.6% and 84.9 ± 0.6% before and after the application of SEA0400, respectively; n = 4) when it was administered after the steady-state constriction was obtained at 60 mmHg. Raising the pressure from 60 to 100 mmHg induced no further myogenic constriction (Fig. 2A), although a small transient [Ca\(^{2+}\)] elevation was observed (Fig. 2B), suggesting that myogenic constriction of rat posterior cerebral arteries is the maximum at the physio-

![Fig. 1. Functional existence of Na\(^+/\)Ca\(^{2+}\) exchanger (NCX) in rat posterior cerebral arteries (PCA). A: NCX1, NCX2, and NCX3 transcripts in rat PCA were analyzed by RT-PCR. Total RNA was isolated from rat PCA or rat whole brain. Ctl indicates samples in the absence of total RNA (negative control). Primers were designed to mammalian NCX1, 2, and 3: NCX1 (5'-GCGGCTTTTAAAAGGCCTGAC-3' and 5'-CTAGACAGAGATGCGCAACGC-3'); NCX2 (5'-GATAGCAAGATGCCAGACACCACGCTCAT-3'); and NCX3 (5'-AGCTGCTCTTCCACACACAG-3' and 5'-GTCACGGACCTTGGTAAGGC-3'). The PCR protocol used started with an initial 15-min denaturation step at 95°C, followed by 30 cycles of the profile consisting of 45 s of denaturation at 94°C, 45 s of annealing at 59°C, and 30 s of extension at 72°C. PCR products were separated by electrophoresis on a 2% agarose gel and visualized by ethidium bromide staining. The data shown are representative of three independent experiments. B: Effect of SEA0400, an NCX inhibitor, on [Ca\(^{2+}\)] elevation in response to Na\(^+\)-free Tyrode’s solution, which was prepared by substituting N-methyl-D-glucamine for Na\(^+\), in the presence of nicardipine (2 μM) at the intraluminal pressure of 10 mmHg in rat posterior cerebral arteries. The isolated arteries were cannulated with two glass pipettes in Tyrode’s solution, which was composed of 158.3 mM NaCl, 4.0 mM KCl, 2.0 mM CaCl\(_2\), 1.05 mM MgCl\(_2\), 10.0 mM NaHCO\(_3\), 0.42 mM NaH\(_2\)PO\(_4\), and 5.5 mM glucose, and the intraluminal pressure was regulated with an electronic pressure servosystem. After the cannulation, the arterial segments were loaded with fura 2-AM. The segments were excited alternately at 340 and 380 nm, and emitted light at 510 nm was measured every 30 s with a fluorescence ratio imaging system. Averaged ratio values from the whole artery image were used to calculate [Ca\(^{2+}\)]. SEA0400 was administered to the superfusing solution 20 min before the measurements. Pretreatment with SEA0400 decreased the peak amplitude of [Ca\(^{2+}\)] elevation induced by Na\(^+\)-free solution in a concentration-dependent manner. SEA0400 was administered to the superfusing solution 20 min before the measurement. Data are each the mean ± S.E.M. of 4 experiments. **P < 0.01 vs. 0 mM SEA0400 (Dunnett’s multiple comparison test). Protocols for animal use were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Shizuoka and were in accordance with guidelines approved by The Japanese Pharmacological Society.](image-url)
Fig. 2. Effect of SEA0400 on pressure-induced constriction (A, D) and $[\text{Ca}^{2+}]$ elevation (B, E) in rat posterior cerebral arteries. Intraluminal pressure was raised from 10 to 60 and 100 mmHg (A, B) and from 10 to 100 mmHg (D, E) in the absence (Control) and presence of SEA0400 (1 μM). Statistical analysis was performed at 0.5 and 10 (C) or 20 min (F) after the elevation of intraluminal pressure. SEA0400 was administered to the superfusing solution 20 min before the measurements. Myogenic constriction was calculated by subtracting the inner diameter in normal Tyrode’s solution from the passive one, which was measured at the end of each experiment in the $\text{Ca}^{2+}$-free Tyrode’s solution containing 2 mM EGTA, and normalized to the maximal constriction induced by 60 mM K$. The endothelium was removed by placing an air bubble in the lumen for 1 min. Data are each the mean ± S.E.M. of 5 experiments. *$P<0.05$, **$P<0.01$; n.s., not significant. (Student’s unpaired $t$-test).
logical intraluminal pressure of 60 mmHg. SEA0400 (1 μM) had no effect on them (Figs. 2: A and B). We confirmed that 60 mM K⁺ caused further constriction and [Ca²⁺]; elevation at the intraluminal pressure of 60 mmHg (data not shown), indicating that the vessels had a capacity to constrict more even at 60 mmHg. These results suggest that in rat posterior cerebral arteries, the Ca²⁺ entry mode of NCX is involved in the development, but not the steady-state, of pressure-induced myogenic constriction. Although the Ca²⁺ entry via NCX seems to be a little activated even in the steady-state, it is unlikely to directly couple to vasoconstriction.

Our previous study has suggested that at least two types of NSCC are involved in the pressure-induced myogenic constriction: one is sensitive to 10 μM ruthenium red (RuR) and involved in the initial development of constriction, and another is inhibitable by 30 μM RuR and the PKCδ inhibitor rottlerin and involved in the steady-state. Mechanical stresses such as intraluminal pressure elevation have been shown to activate TRPC6 activated by ATP, which in turn activates the Ca²⁺ entry mode of NCX (5). The initial development of myogenic constriction and [Ca²⁺]; elevation was inhibited by SEA0400 in the present study. It is thus likely that Na⁺ entry through RuR-sensitive, stretch-activated NSCC drives the Ca²⁺ entry mode of NCX, which results in the initial rise in [Ca²⁺].

The results of [Ca²⁺]; measurement with SEA0400 suggest that Ca²⁺ entry via NCX contributes to not only the initial phase of pressure-induced [Ca²⁺]; elevation but also the sustained one. However, the Ca²⁺ entry in the sustained phase did not couple to vasoconstriction. This discrepancy might be explained by the localization of [Ca²⁺]; elevation. The Ca²⁺ entry mode of NCX has been shown to increase Ca²⁺ concentration in the cytosolic microdomain immediately beneath the plasmalemma in vascular smooth muscle cells (14). The localized rise in [Ca²⁺]; might be unable to lead to the activation of myosin light chain kinase to cause vasoconstriction.

We have previously shown that conventional PKC (cPKC) contributes to the development of pressure-induced myogenic constriction by mediating Ca²⁺ sensitization in rat posterior cerebral arteries (8). The myogenic constriction in the presence of the cPKC inhibitor Gö6976 was slowly developing, which is similar to that in the presence of SEA0400 shown in the present study. It is thus likely that Ca²⁺ entry via NCX activates cPKC, thereby inducing Ca²⁺ sensitization during the development of myogenic constriction. NCX-mediated Ca²⁺ influx has been suggested to activate PKC in Ca²⁺-preconditioned hearts (15). Of interest is that the [Ca²⁺]; elevation via the Ca²⁺ entry mode of NCX may not directly affect myosin function not only in the steady-state but also in the development of myogenic constriction. Further studies are required to clarify the relationships between cPKC and NCX and between Ca²⁺ entry via NCX and vasoconstriction.

The current data suggest that NCX-mediated Ca²⁺ entry contributes little to physiological regulation to maintain cerebral artery myogenic constriction. In contrast to the present results, the inhibition of NCX activity impaired both the development and steady-state of myogenic constriction in rat cremaster muscle arterioles (7). The contribution of the Ca²⁺ entry mode of NCX to steady-state myogenic constriction may be different between cerebral and peripheral vessels. Since the steady-state myogenic constriction at physiological intraluminal pressure was accompanied with a relatively high [Ca²⁺]; level in rat cerebral arteries, the functional activity of the Ca²⁺ entry mode of NCX may be reduced by it (3). Possible contribution of the Ca²⁺ entry mode of NCX to myogenic constriction of cerebral arteries would be expected to occur in pathological conditions such as recirculation following cerebral ischemia.

In summary, we demonstrate functional activity of the Ca²⁺ entry mode of NCX in the development, but not in the steady-state, of pressure-induced myogenic constriction in rat posterior cerebral arteries. Our data imply that rapid elevation of intraluminal pressure induces Na⁺ influx via stretch-activated NSCC, which drives NCX-mediated Ca²⁺ entry, and that the Ca²⁺ entry mode of NCX is continuously active although it does not contribute to vasoconstriction in cerebral arteries.

References


