Role of Cl\textsuperscript{−} current in endothelin-1-induced contraction in rabbit basilar artery

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ION FLUX AND FLUORESCENT DYE STUDIES have demonstrated that the intracellular Cl\textsuperscript{−} concentration ([Cl\textsuperscript{−}]\textsubscript{i}) is in the range of 39–107 mM and accumulates by at least three processes, including Na\textsuperscript{+}-K\textsuperscript{+}-2Cl\textsuperscript{−} cotransporter, Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange, and ATP-dependent transporter pump III (4). The equilibrium potential for Cl\textsuperscript{−} (\(E_{Cl}\)), calculated by 60 log [Cl\textsuperscript{−}]\textsubscript{i} and extracellular Cl\textsuperscript{−} concentration ([Cl\textsuperscript{−}]\textsubscript{o}), is between −20 and −30 mV, which is roughly 15–30 mV more positive than the resting membrane potential. Consequently, the opening of Cl\textsuperscript{−} channels causes Cl\textsuperscript{−} efflux, drives the membrane potential toward \(E_{Cl}\), depolarizes the cell membrane, and contracts smooth muscle cells. Cl\textsuperscript{−} channels are found in many types of cells, including portal venous cells (20), ear artery cells (2), and esophageal smooth muscle cells (1). Three kinds of Cl\textsuperscript{−} channels were identified in smooth muscle cells: voltage-dependent large-conductance Cl\textsuperscript{−} channels (33), volume-sensitive Cl\textsuperscript{−} channels (13), and Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} channels. Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels are the most frequently studied Cl\textsuperscript{−} channels. Many agonists, including norepinephrine (NE), methacholine, and histamine, also activate Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels (15, 16, 32). These agonists release Ca\textsuperscript{2+} from the intracellular Ca\textsuperscript{2+} store, which, in turn, activates the Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} current, thus depolarizing the cell membrane and ultimately resulting in contraction. Elevation of intracellular Ca\textsuperscript{2+} also activates Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels, which in turn hyperpolarize membrane potentials. The type of channel that plays a dominant role decides the outcome, that is, either contraction by the activation of Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} channels or relaxation by the activation of Ca\textsuperscript{2+}-dependent K\textsuperscript{+} channels. Cl\textsuperscript{−} channel blockers, i.e., niflumic acid, 4,4-diisothiocyanostilbene-2,2′-disulfonic acid (DIDS), and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB), have been shown to inhibit NE or phenylephrine-induced Ca\textsuperscript{2+}-dependent Cl\textsuperscript{−} current in rat portal vein (20) and contraction of rat pulmonary arteries (18, 20, 29).

Endothelin-1 (ET-1) causes profound vasoconstriction in both arterial and venous smooth muscle. Because ET-1 is a potent and long-lasting vasoconstrictor, it has been regarded as a mediator of vasospasm and hypertension (39, 40). Activation of the ETA receptors in vascular smooth muscle cells exerts the well-recognized contractile effect of ET-1 by inducing depolarization and resulting in increased intracellular Ca\textsuperscript{2+} because of either mobilization of \(\text{d-my}\text{o-inositol 1,4,5-trisphosphate-sensitive Ca}^{2+}\) store or an influx of extracellular Ca\textsuperscript{2+} via dihydropyridine-sensitive Ca\textsuperscript{2+} channels. Increased Ca\textsuperscript{2+} not only leads to a contractile response but might also activate Ca\textsuperscript{2+}-dependent ion channels (27), including Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels such as in rat pulmonary arterial smooth muscle cells (31), porcine coronary arteries (20), human mesenteric arteries (32), and rat renal resistance arteries (11).

Ca\textsuperscript{2+}-activated Cl\textsuperscript{−} channels have been identified in rabbit cerebral artery by electrophysiology (18), although their contribution to contraction in cerebral arteries by agonists such as ET-1 remains undetermined. The current investigation was conducted to investigate whether Cl\textsuperscript{−} channels play a role in the ET-1-induced contraction in the rabbit basilar arteries. The
from 30 to 50 μM acetylcholine (rings precontracted with 30 μM 5-hydroxytryptamine).

The low-Cl\(^-\) solution was prepared by the replacement of 120 mM NaCl with 120 mM NaOH. The pH of the buffer was titrated to 7.4 with the use of methanesulfonic acid while the solution was prewarmed and preaerated.

In all experiments, while the [Cl\(^-\)] was being changed, the low-level Cl\(^-\) solution was prewarmed and preaerated in a 37°C water bath, mixed with the agonists at the designated concentration, and injected slowly into the water bath. The control group with normal Cl\(^-\) solution was done in the same way. The Ca\(^{2+}\)-free solution was prepared by omitting the 2.5 mM Ca\(^{2+}\) and adding 2 mM EGTA. The Ca\(^{2+}\)-free low-Cl\(^-\) solution was prepared by the omission of Ca\(^{2+}\), and the extracellular Cl\(^-\) was reduced as mentioned before. The HEPES buffer was made by replacing 27 mM NaHCO\(_3\) with 20 mM HEPES and titrating the pH to 7.4 using 1 M NaOH solution. The measured osmolality of the normal Cl\(^-\) buffer and the low-[Cl\(^-\)] buffer was 284 and 288 osmol/kgH\(_2\)O, respectively, and the osmolality of the Ca\(^{2+}\)-free buffer was 280 osmol/kgH\(_2\)O.

**Chemicals**

ET-1 was purchased from Alexis; stock solution was 10\(^{-5}\) M in distilled water. NPPB, a nonselective Cl\(^-\) channel blocker, was from Tocris; stock solution was 10\(^{-2}\) M in dimethyl sulfoxide (DMSO). Niflumic acid, a Ca\(^{2+}\)-dependent Cl\(^-\) channel blocker, was purchased from Sigma; stock solution was 0.1 M in DMSO. Indanyloxyacetic acid (IAA-94), a nonselective Cl\(^-\) channel blocker, was purchased from Alexis; stock solution was 10\(^{-2}\) M in DMSO. Bumetanide, a Na\(^{+}\)-K\(^+\)-2Cl\(^-\) cotransporter inhibitor, was purchased from Sigma; stock solution was 10\(^{-2}\) M in ethyl alcohol. The dosage of DMSO and ethyl alcohol used was <0.1% and produced no effects on the resting tension.

**Statistics**

All data are displayed as means ± SE, and n represents the number of arterial rings in each group. One-way analysis
of contraction. Initial contraction of an arterial ring in response to ET-1 at $10^{-8}$ M in normal Cl$^-$ solution (123 mM Cl$^-$) is shown. The experiment was done by prewarming and preaerating the low-Cl$^-$ solution, mixing ET-1 at desired $10^{-8}$ M concentration. The control group (ET-1 in normal Cl$^-$ solution) was done in the identical way.

of variance and $t$-test were used as analysis methods, and a value of $P < 0.05$ was considered to be significantly different.

RESULTS

ET-1-Induced Contraction in Rabbit Basilar Artery

In several previous reports (40), we have established that ET-1 produces concentration-dependent contraction in rabbit basilar arteries by activating the ETA receptor. The ETA receptor antagonist BQ-610, but not the ETB receptor antagonist BQ-788, reduced the contraction to ET-1. The EC$_{50}$ and maximum normalized response (to 120 mM KCl) of ET-1 from 0.1 to 100 nM are 8.10 ± 1.54 nM and 199 ± 2.45%, respectively. The EC$_{50}$ and maximum normalized response (to 120 mM KCl) of ET-1 in the presence of bumetanide at 100 µM are 10.5 ± 1.18 nM and 87.2 ± 3.14%, respectively.

Effect of Cl$^-$ Channels and ET-1

Rings were pretreated in HEPES solution (2 × $10^{-2}$ M, HCO$_3^-$ free, $n = 5$) for 30 min before application of ET-1 from $10^{-10}$ to $10^{-7}$ M. The HEPES solution significantly suppressed the contraction induced by ET-1 (Fig. 2). The EC$_{50}$ and maximum normalized response (to 120 mM KCl) of ET-1 in the presence of HEPES solution (HCO$_3^-$/Cl$^-$ exchanger inhibitor) were 1.94 ± 0.16 nM and 98.5 ± 1.40%, respectively.

Effect of Decreasing [Cl$^-$]$_o$ on ET-1-Induced Contraction

Rings were contracted first in a normal 123 mM Cl$^-$ solution by ET-1 at $10^{-8}$ M for at least 20 min. After tension attained the stable and plateau phase, the normal Cl$^-$ buffer was quickly drained and exchanged for either a fresh normal Cl$^-$ buffer (123 mM Cl$^-$) or low-Cl$^-$ (methanesulfonate ion inhibitor) for 30 min before application of ET-1 from $10^{-10}$ to $10^{-7}$ M. Bumetanide significantly suppressed the contraction in a concentration-dependent and reversible manner (Fig. 1). The EC$_{50}$ and maximum normalized response (to 120 mM KCl) of ET-1 in the presence of the Na$^+$-K$^+$-2Cl$^-$ cotransporter inhibitor bumetanide at 30 µM are 11.1 ± 0.68 nM and 109 ± 2.45%, respectively. The EC$_{50}$ and maximum normalized response (to 120 mM KCl) of ET-1 in the presence of bumetanide at 100 µM are 10.5 ± 1.18 nM and 87.2 ± 3.14%, respectively.

Effect of Cl$^-$ /HCO$_3^-$ Exchange on ET-1-Induced Contraction

Rings were pretreated in HEPES solution (2 × $10^{-2}$ M, HCO$_3^-$ free, $n = 5$) for 30 min before application of ET-1 from $10^{-10}$ to $10^{-7}$ M. The HEPES solution significantly suppressed the contraction induced by ET-1 (Fig. 2). The EC$_{50}$ and maximum normalized response (to 120 mM KCl) of ET-1 in the presence of HEPES solution (HCO$_3^-$/Cl$^-$ exchanger inhibitor) were 1.94 ± 0.16 nM and 98.5 ± 1.40%, respectively.
Ca\(^{2+}\)-free solution. A more concentrated solution of ET-1 (10\(^{-7}\) M vs. 10\(^{-8}\) M) in a Ca\(^{2+}\)-free buffer was used to achieve a similar degree of contraction (of ET-1 observed when normal Cl\(^{-}\) solution was changed to fresh normal Cl\(^{-}\) solution (Fig. 4A). In contrast, tension was markedly enhanced when low-Cl\(^{-}\) buffer was used (Fig. 4B). Figure 5 summarized the effect of low-Cl\(^{-}\) buffer on the initiation and sustained contraction of ET-1.

Effects of Cl\(^{-}\) Channel Blockers on ET-1-Induced Contraction

Preincubation with Cl\(^{-}\) channel blockers. Rings were pretreated with niflumic acid, NPPB, and IAA-94 at 3 x 10\(^{-5}\) or 10\(^{-4}\) M, respectively, for 30 min before ET-1 applications in the range of 10\(^{-10}\)–10\(^{-7}\) M. The Cl\(^{-}\) channel blockers had no effect on the resting tension. Pretreatment with niflumic acid, NPPB, and IAA-94 attenuated ET-1-induced contraction in a concentration-dependent and reversible manner (Fig. 7).

Relaxant effect of Cl\(^{-}\) channel blockers. ET-1 (10\(^{-8}\) M) was used first to achieve a sustained contraction. The relaxant effect of niflumic acid, NPPB, and IAA-94 was tested in solutions in the range of 10\(^{-6}\)–10\(^{-4}\) M. All inhibitors induced a concentration-dependent relaxation. Figure 8 shows the original tracing of the effect of Cl\(^{-}\) channel blockers. Figure 9 summarizes the dose-dependent effect of IAA-94 (n = 12), NPPB (n = 10), and niflumic acid (n = 7) on ET-1 (10\(^{-8}\) M)-elicited contraction on endothelium intact rings. The 50% inhibitory concentration (IC\(_{50}\)) values of the Cl\(^{-}\) channel blockers NPPB, IAA-94, and niflumic acid on ET-1-induced contractions averaged 39.0 ± 2.96, 43.9 ± 6.81, and 58.6 ± 8.54 μM, respectively.

DISCUSSION

Our present study supports the hypothesis that Cl\(^{-}\) channels are involved in ET-1-induced contraction and modulation of Cl\(^{-}\) channels alters ET-1-induced contraction in cerebral arteries. The evidence established by this study is as follows. First, the Na\(^{+}\)-K\(^{-}\)-2Cl\(^{-}\) cotransporter inhibitor bumetanide depresses the ET-1-induced contraction. Second, the HCO\(_3\)\(^{-}\) -free solution (HEPES) inhibits ET-1-induced contraction. Third, low-extracellular Cl\(^{-}\) solution enhances both initial and sustained phases of contraction induced by ET-1. Extracellular Ca\(^{2+}\) influx causes the potentiation effect of low-extracellular Cl\(^{-}\). Fourth, pretreatment with NPPB, niflumic acid, and IAA-94 attenuates the ET-1-induced contraction in endothelially intact rings. Fifth, the Cl\(^{-}\) channel blockers NPPB, niflumic acid, and IAA-94 relax the sustained ET-1-induced contraction in endothelially intact rings.

Role of Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) Cotransporter and Cl\(^{-}\)/HCO\(_3\)\(^{-}\) Exchanger in ET-1-Induced Contraction

The Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter is one of three mechanisms responsible for the accumulating intracellular Cl\(^{-}\) in smooth muscle cells (23). The receptor agonist NE activates the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter to increase intracellular Cl\(^{-}\) and depolarizes the membrane potential in rat femoral arterial smooth muscle cells (7, 21). Bumetanide, a Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\} cotrans-
porter inhibitor, decreases intracellular Cl\(^{-}\) and hyperpolarizes membrane potential. Bumetanide relaxes NE-induced contractions in the rat aorta (7, 21). Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter activity is more active in arterial smooth muscle in the deoxycorticosterone acetate/salt model of hypertension than in normotensive control rats (4, 8). Consequently, the level of intracellular Cl\(^{-}\) is higher, and the E\(_{\text{Cl}}\) is more positive in hypertension, thus contributing to the hypertensive depolarization and maintenance. Those studies indicate that the Na\(^{+}\)-K\(^{+}\)-2Cl\(^{-}\) cotransporter plays a role in vascular smooth muscle cells, which modulates the membrane potential and contraction. Our study

Fig. 6. Potentiating effect of low-Cl\(^{-}\) buffer dependents on extracellular Ca\(^{2+}\). A: typical response of an arterial ring to ET-1 at 10\(^{-7}\) M in Ca\(^{2+}\)-free 123 mM Cl\(^{-}\) buffer during the sustained contraction phase. Changing solution (arrow) to Ca\(^{2+}\)-free 10 mM Cl\(^{-}\) buffer depressed ET-1-induced contraction. B: summarization of the effect of low-Cl\(^{-}\) in the absence of extracellular Ca\(^{2+}\) buffer. *P < 0.05 vs. Ca\(^{2+}\)-free normal Cl\(^{-}\) buffer, one-way ANOVA.

Fig. 7. Original traces of Cl\(^{-}\) channel inhibitors on 10\(^{-8}\) M ET-1-induced contraction on endothelium intact rings. A–C: typical response of an arterial ring to ET-1 at 10\(^{-8}\) M after application of niflumic acid, 5-nitro-2-(phenylpropylamino)benzoic acid (NPPB), and indanyloxyacetic acid 94 (IAA-94) from 10\(^{-6}\) to 10\(^{-4}\) M. Numbers with arrows indicate accumulated dosage (in \(\mu\)M) of the Cl\(^{-}\) channel inhibitors niflumic acid, NPPB, and IAA-94.

Fig. 8. Cl\(^{-}\) channel inhibitors IAA-94, NPPB, and niflumic acid relax the ET-1 (10\(^{-8}\) M)-induced contraction in a concentration-dependent manner on endothelium intact rings. IAA-94, NPPB, and niflumic acid from 10\(^{-8}\) to 10\(^{-4}\) M were applied to the chamber after obtaining a sustained contraction to 10\(^{-8}\) M ET-1. *P < 0.05 vs. control, one-way ANOVA.
showed that bumetanide depresses ET-1-induced contraction and consistently demonstrated that Cl- current and the Na+-K+-2Cl- cotransporter are involved in the ET-1 contractile response in cerebral arteries.

It has been reported that except for its function of modulating intracellular pH, the Cl-/HCO3- exchanger also plays a role in the accumulation of intracellular Cl- above EClin. Accumulating Cl- via the Cl-/HCO3- exchanger appears to occur independently of the Na+-K+-2Cl- cotransporter; moreover, the two effects are additive (6). The bicarbonate-free solution depresses NE-induced contraction in rat aortas, thus indicating the functional role of the Cl-/HCO3- exchanger in vascular smooth muscle tissue (21). Evidence in our study showing that the HCO3- free solution inhibits ET-1-induced contraction in the rabbit basilar arteries is consistent with this view and demonstrates that Cl-/HCO3- exchanger contributes to cerebral arterial contraction. Although the Na+-H+ exchanger might possibly alter the pH and/or volume, our study showed that the HCO3- free solution affected neither the resting tension nor the 60 mM KCl-induced contraction (unpublished communications), thereby indicating arterial contractile function is intact.

Role of [Cl-], in ET-1-Induced Contraction

The methanesulfonate ion is an impermeant Cl- channel anion. Because of its negligible permeability and apparent lack of interaction with Cl-, it is used as the preferred impermeant Cl- substitute in substitution studies of membrane conductance (21, 30). We found that both at the initial and sustained contraction phases, the low-Cl- solution significantly enhanced ET-1-induced contraction compared with contraction in the normal Cl- solution. This enhanced contraction in low-Cl- solution disappeared when the normal Cl- and Ca2+-free solution was changed to the low-Cl- and Ca2+-free solution. This study suggests that the low [Cl-]c might induce Cl- efflux and depolarization while triggering Ca2+ entry from the extracellular space. The alteration of EClin is probably the mechanism of the potentiating effect of a low-Cl- buffer. Given the assumption that the internal Cl- is 54 mM (the value in the rabbit choroid smooth muscle cells) (5) and that the extracellular Cl- is normally 123 mM, as used in this study, extracellular Cl- decreasing from 123 to 10 mM results in membrane depolarization. Other investigators have also observed enhanced pressure-induced myogenic tone (26) and that a low-Cl- buffer potentiates NE-induced contraction in rat aortas (21). In contrast, a Cl- free buffer inhibits phenylephrine-induced contraction and Ca2+ influx in the rat caudal artery (33). Variation in experimental procedure or contractile mechanism of the caudal artery might possibly explain these differences. In the rat aorta, we exposed low-Cl- solution and NE to the arterial rings at the same time by premixing NE with the low-Cl- buffer (21). Under such circumstances, the intracellular Cl- was not depleted (21). However, in rat caudal artery preparations (33), the arterial rings were washed with Cl- free buffer and allowed to equilibrate for 30 min in Cl- free solution before application of phenylephrine. At the time that phenylephrine was administered, the intracellular Cl- had already been depleted. The differences in the two experimental conditions might explain the contradictory responses to the low-Cl- solution by NE and phenylephrine.

Low Cl- has caused some minor potentiation differences between NE-induced contraction and ET-1-induced contraction. Low Cl- buffer enhances NE-induced contraction more markedly at the initial contraction point than during the sustained contraction phase (21). In contrast, low-Cl- buffer enhances ET-1 markedly during both the initial contraction and the sustained contraction.
contraction, indicating a different contractile response mechanism with respect to these agonists. Recent studies (25) have demonstrated that concentrations of ET-1 >10^{-8} M activate both nonselective cation channels and store-operated Ca^{2+} channels. The voltage-dependent Ca^{2+} channel plays a minor role in ET-1-induced contraction but plays a major role in NE-induced contraction (12, 25). Furthermore, in the microvascular smooth muscle cells of choroidal arteries, Cl^- channels participate in ET-1-induced activation of the store-depletion-dependent Ca^{2+} channel, and Cl^- serves to protect smooth muscle cells from Ca^{2+} overload (5).

Another issue is the “long-term” effect of Cl^- channels, especially the Ca^{2+}-activated Cl^- channels operating in smooth muscle contraction. After intracellular Ca^{2+} levels rise, Ca^{2+}-activated Cl^- channels activate and decay rapidly in smooth muscle cells (28). Channel inactivation, resulting from phosphorylated channel protein by calcium/calmodulin-dependent kinase II, terminates the Ca^{2+}-activated Cl^- channel (37). Thus, to prolong the effect of Cl^- channels in enhancing ET-1-induced cerebral arterial contraction, other Cl^- channels might need to be involved.

Role of Cl^- Channel Blockers in ET-1-Induced Contraction

Although their functional roles and expressions remain controversial, Ca^{2+}-activated Cl^- channels are the most frequently studied Cl^- channels in smooth muscle cells. Using isolated cerebral arteries, Nelson et al. (26) suggested that Cl^- channels play a role in pressure-induced cerebral arterial myogenic tone and that the nonselective Cl^- channel blockers IAA-94 and DIDS displayed dilated pressurized rat cerebral arteries. However, Nelson et al. (26) reported that the Ca^{2+}-activated Cl^- channel blocker niflumic acid failed to show any inhibitory effect on the developed tone. Several groups of investigators studied the possible function of Ca^{2+}-activated Cl^- channels in cerebral arteries and reported contradictory results. When Salter et al. (31) used single smooth muscle cells from rat posterior cerebral arteries, ET-1 or photorelease of caged Ca^{2+} failed to evoke inward current, thus suggesting either that rat cerebral arteries do not possess Ca^{2+}-activated Cl^- channels or that Ca^{2+}-activated Cl^- channels do not play a major role. Kamouchi et al. (17), however, used the perforated patch technique and histamine to trigger Ca^{2+}-activated Cl^- currents in rabbit basilar arteries. The expression of Ca^{2+}-activated Cl^- channels and their role in modulating membrane potential in cerebral smooth muscle cells might be species and tissue specific. In the current study, although the Ca^{2+}-activated Cl^- channel blocker niflumic acid reduces the contraction of rabbit basilar arteries exposed to ET-1 and relaxes the sustained contraction induced by ET-1, whether Ca^{2+}-activated Cl^- channels mediated the effect of niflumic acid remains yet to be determined. Because the other Cl^- channel inhibitors used in this study are nonselective, the involvement of other Cl^- channels or other ionic chan-

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**Smooth muscle cells**

Fig. 10. Schematic summary of proposed mechanism of ET-1-induced contraction in cerebral arteries. ET-1 activates ET_{A} receptor (R) and generates d-myo-inositol 1,4,5-trisphosphate (IP_{3}), which that releases Ca^{2+} from internal stores through IP_{3} receptors (IP_{3}R). Elevation of internal Ca^{2+} may trigger Ca^{2+} release from ryanodine receptors (RyR) as Ca^{2+}-induced Ca^{2+} release. Elevation of internal Ca^{2+} opens Ca^{2+}-activated or other types of Cl^- channels that may be blocked by IAA-94, NPPB, and niflumic acid. Opening of Cl^- channels depolarizes membrane potential and activates voltage-dependent Ca^{2+} channels (VDCC) and results in Ca^{2+} entry. The Na+-K+-2Cl^- cotransporter and HCO_{3}/Cl^- exchanger can be blocked by bumetanide and HEPES. Inhibition of Na+-K+-2Cl^- cotransporter and HCO_{3}/Cl^- exchanger will reduce Cl^- accumulation and hyperpolarizes membrane potential.
nels, such as Ca\(^{2+}\) channels, cannot be excluded. In the current study, we used the [Cl\(^{-}\)] channel blockers that were reported to selectively block Cl\(^{-}\) channels in other studies (9, 23, 26). For example, in rat cerebral arteries (26), IAA-94 at 300 \(\mu\)M relaxed myogenic response by 92 \(\pm\) 6\% (IC\(_{50}\) = 26 \(\pm\) 1.5 \(\mu\)M). NPPB (at 100 \(\mu\)M) completed blocked 45 mM K\(^{+}\) depolarization-induced isometric tension (IC\(_{50}\) = 10.0 \(\pm\) 0.76 \(\mu\)M). IAA-94 (200 \(\mu\)M) completed relaxed 45 mM K\(^{+}\) depolarization-induced isometric tension (IC\(_{50}\) = 17.0 \(\pm\) 1.2 \(\mu\)M) (9). In pulmonary arterial rings, niflumic acid and NPPB relaxed ET-1 (30 nM)-induced contraction (IC\(_{50}\) = 55.8 \(\mu\)M for niflumic acid and IC\(_{50}\) = 21.1 \(\mu\)M for NPPB). Because there is no evidence for a direct coupling between Cl\(^{-}\) channels and ET\(_{A}\) receptors in the cerebral arteries, and the Na\(^{+}\)-K\(^{+}\)-2Cl cotransporter inhibitor bumetanide, the HCO\(_{3}\)/Cl\(^{-}\) exchanger inhibitor HEPEs solution, and the Cl\(^{-}\) channel blockers are not the competitive antagonists to ET\(_{A}\) receptors, the antagonist affinity value equilibrium dissociation constant is not reported here.

Although several investigative groups reported that many compounds might block the Cl\(^{-}\) current, none of those compounds is selective for a particular subtype of Cl\(^{-}\) current. Previously, Doughty et al. (9) and Toma et al. (35) indicated that the inhibitory effect of Cl\(^{-}\) channel blockers, such as niflumic acid, operate by opening K\(^{+}\) channels. Furthermore, Cl\(^{-}\) channel blockers directly affect voltage-dependent Ca\(^{2+}\) channels in isometric tension and patch-clamp studies. Kato et al. (18) reported that NPPB and niflumic acid relax ET-1-induced contraction in pulmonary arteries but that the mechanism is independent of the Cl\(^{-}\) channel blocking action. Doughty et al. (9) observed a direct inhibitory effect of NPPB and IAA-94 on KCl-induced contraction in rat cerebral artery. These reports indicate that the relaxant effect of Cl\(^{-}\) channel blockers is nonselective and at least partially results from blocking the voltage-dependent Ca\(^{2+}\) channels. The endothelium is another possible source of the “nonselective” relaxant effect of the Cl\(^{-}\) channel blockers. Vasoactive agents released from the endothelium regulate vascular tone in cerebral arteries. In a recent study, Lamb et al. (22) indicated that nitric oxide released from endothelial cells might regulate both the resting Cl\(^{-}\) conductance and the ability of agonists to activate the Cl\(^{-}\) channel.

**Perspective**

To our knowledge, this is the first investigation of the effect of Cl\(^{-}\) channels in ET-1-induced contraction in cerebral arteries. We used various approaches to demonstrate that modulation of Cl\(^{-}\) channels alters ET-1-induced contraction in rabbit basilar arteries. We use in a schematic diagram to summarize the role of Cl channel in ET-1-induced contraction (Fig. 10). ET-1 activates ET\(_{A}\) receptors and, by unknown mechanisms, ET-1 activates the Ca\(^{2+}\)-activated or other Cl\(^{-}\) channels, all of which result in contraction. Varying Cl\(^{-}\) levels, intracellularly or extracellularly, affect ET-1-induced contraction. The Cl\(^{-}\) channel inhibitors NPPB, IAA-94, and niflumic acid attenuate ET-1-induced contraction.

In conclusion, the mechanism regulating Cl\(^{-}\) current responses to ET-1, however, remains unknown, although it is presumed that an elevation of intracellular Ca\(^{2+}\), released either from internal stores or drawn from extracellular space, might initiate some Cl\(^{-}\) channel activation, especially the Ca\(^{2+}\)-activated Cl\(^{-}\) channels. The possible involvement of other Cl\(^{-}\) channels and how they are involved remains to be explored. The interaction between Cl\(^{-}\) channels and Ca\(^{2+}\) or K\(^{+}\) channels after ET-A receptor activation warrants further study. A clear understanding of the mechanism of ET-1-induced contraction is critical to some clinical situations such as cerebral vasospasm and hypertension, in which ET-1 is involved (3, 41).

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