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REVIEW —*Current Perspective*—

Chloride Channels and Their Functional Roles in Smooth Muscle Tone in the Vasculature

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ABSTRACT—Although evidence of important contributions by Cl^- channels to agonist-induced currents have been reported in vascular smooth muscle cells, the functional roles played by Cl^- channels in the smooth muscle contraction and in setting the membrane potential remain essentially obscure. All of the admittedly few papers published have focused on the physiological roles of Cl^- channels in the contraction and membrane depolarization elicited by agonists. At present, it seems likely that in vascular cells: a) Cl^- conductance contributes to membrane depolarization, with the subsequent contraction being due to Ca^{2+} release from the intracellular store sites, and b) Cl^- movements through the membrane of the Ca^{2+} store sites also regulate Ca^{2+} release and Ca^{2+} uptake from/into the store sites. As a Ca^{2+}-dependent Cl^- current is most easily demonstrated under quasi-physiological conditions (by the perforated patch-clamp method), the contribution made by Cl^- channels to smooth muscle function may be more important than previously thought. The development of the new, selective Cl^--channel blockers as well as the identification and gene engineering of the channel molecules are essential if we are to advance our knowledge of the physiology and pharmacology of the Cl^- channels residing in vascular smooth muscle cells.

Keywords: Ca^{2+}-dependent Cl^- channel, Artery, Smooth muscle, Cl^--dependent contraction, Cl^--dependent depolarization

Introduction

It is well known that L-type Ca^{2+} channels are important in the regulation of the intracellular Ca^{2+} concentration in vascular smooth muscle cells, and some other channels, such as voltage- and ligand-gated K^+ channels or nonselective cation channels, are known to regulate the L-type Ca^{2+} channels through changes in the membrane potential. In contrast, we still know comparatively little about the other type of ion channel that is abundantly distributed in the smooth muscle membrane, the Cl^- channel. The Cl^- conductance through the membrane of smooth muscle cells is higher than the corresponding conductances in skeletal and cardiac muscle cells. In rabbit pulmonary arterial cells, an intracellular Cl^- concentration of 57 mM has been calculated, and the relative permeability for Cl^- is 0.67 against the K^+ permeability under resting membrane conditions (1). The calculated equilibrium potential for Cl^- in these vascular cells is −26 mV (1), which is a high enough potential to activate the L-type Ca^{2+} channels in these cells, and also high enough to block the spontaneously generated action potential in excitable cells (depolarization block). This high Cl^- conductance may be an important determinant of the indigenous membrane properties of smooth muscle cells. In actual fact, an increase in Cl^- conductance produces a bidirectional response in terms of smooth muscle contraction. In vascular smooth muscle cells, which (with the exception of the portal vein) do not generate an action potential spontaneously in the presence or absence of agonists, an increase in Cl^- conductance may contribute to muscle contraction. Thanks to the use of voltage-clamp methods, much evidence has accumulated to demonstrate the presence of Cl^- channels in vascular smooth muscle and to elucidate their electrophysiological and pharmacological properties, but it is still not yet clear exactly how Cl^- channels contribute to the regulation of smooth muscle contraction and membrane depolarization elicited by various agonists. An adequate knowledge of the current view of the properties of Cl^- channels or Cl^- conductance, as they relate to the regulation of contraction, is essential for an understanding of their physiological and pathophysiological roles. The present paper reviews the present situation with regard to our

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knowledge of Cl^--channel physiology and pharmacology in vascular smooth muscle cells.

Intracellular Cl^- concentration

The intracellular Cl^- concentration ($[Cl^-]_i$) has been measured in a number of tissues and cells using a variety of methods. Using several different methods, such as the double-barrelled microelectrode method, electron microprobe analysis and Cl^--flux measurement using a radioactive tracer, we were able to measure $[Cl^-]_i$ in smooth muscle tissues. However, as smooth muscle tissues have a relatively large extracellular space, the values obtained for $[Cl^-]_i$ by the use of these classical methods are subject to errors. With the recent development of Cl^--sensitive fluorescent dyes (MQAE and SPQ), we are now able to measure $[Cl^-]_i$ in cells and tissues in which measurement was difficult in the past. However, due to the chemical properties of these dyes (high permeability through the membrane), this method, too, has limitations in terms of the precise measurement of $[Cl^-]_i$ in tissue preparations.

In smooth muscle cells, $[Cl^-]_i$ is reported to be ca. 50 mM, as measured by tracer and ion-selective electrode methods (1 – 4). These values are somewhat larger than that measured in cardiac cells (5 – 8) (Table 1) and that

Table 1. Intracellular Cl^- concentrations in various cells

Tissue	Cell	Species	Intracellular Cl^- concentration (mM)	Tools	References
Smooth muscle tissue	pulmonary artery	rabbit	51	radioisotope	1
	aorta	rat	31.6 ± 3.2	fluorescent dye (MQAE)	8
	vas deferens	guinea pig	53.9	radioisotope	2
	taenia caeci	guinea pig	49 – 56	radioisotope	3
	ureter	guinea pig	51.1	ion-selective electrode	4
Cardiac tissue	ventricular cell	rat	21.3 ± 1.5	ion-selective electrode	5
	ventricular cell	guinea pig	18.7 ± 3.5	ion-selective electrode	6
	heart	chick	25.1 ± 7.3	radioisotope	7
	papillary muscle	rabbit	15.2 ± 0.6 (bicarbonate) 24.1 ± 0.6 (HEPES)	radioisotope	8
Epithelial tissue	aortic endothelial cell	human	34	patch clamp	a)
	tracheal epithelial cell	dog	43 ± 4	fluorescent dye (SPQ)	b)
	corneal epithelium	rabbit	30, 41	microelectrode	c), d)
	renal epithelial cell	rat	28	electron probe analysis	e)
	proximal tubule	rabbit	17.8 ± 0.5	ion-selective electrode	f)
Neurons & others	olfactory cell	newt	40	fluorescent dye (MQAE)	g)
	brain synaptosome	rat	14 ± 4	fluorescent dye (MQAE)	h)
	Dieter's neuron	rabbit	21	radioisotope	i)
	cochlear hair cell	guinea pig	8.3 ± 0.9	fluorescent dye (MQAE)	j)
	epididymal cell	rat	62.3 ± 0.2	fluorescent dye (SPQ)	k)
	kidney cortical thick ascending limb	rabbit	58.8 ± 7.2	fluorescent dye (SPQ)	l)
	kidney macula densa	rabbit	68.7 ± 9.8	fluorescent dye (SPQ)	l)
	gallbladder	Nect-urus	12 ± 2	ion-selective electrode	m)
	red blood cell	lamprey	57.6 ± 5.2	ion-selective electrode	n)
	lymphocyte	rat	70 – 85	radioisotope	o)
	parotid acinar cell	rat	62.3	radioisotope	p)
	fibroblast	human	53.4 ± 3.4	fluorescent dye (MQAE)	q)
	rectotroph cell	rat	59.4	fluorescent dye (SPQ)	r)

a) Ono et al.: J Physiol **511**, 837 – 849 (1998); b) Chao et al.: J Memb Biol **113**, 193 – 202 (1990); c) Klyce & Wong: J Physiol (Lond) **266**, 777 – 799 (1977); d) Yasukura et al.: Jpn J Pharmacol **67**, 315 – 320 (1995); e) Larsson et al.: Acta Physiol Scand **126**, 321 – 332 (1986); f) Ishibashi et al.: Am J Physiol **255**, F49 – F56 (1988); g) Nakamura et al.: Neurosci Lett **237**, 5 – 8 (1997); h) Engblom & Åkerman: Biochim Biophys Acta **1153**, 262 – 266 (1993); i) Rapallino et al.: Int J Neurosci **53**, 135 – 141 (1990); j) Ohnishi et al.: Am J Physiol **263**, C1088 – C1095 (1992); k) Huang et al.: J Physiol (Lond) **474**, 183 – 194 (1994); l) Salomomsson et al.: Acta Physiol Scand **147**, 305 – 313 (1993); m) Fernando-Garcia-Diaz et al.: J Membr Biol **73**, 145 – 155 (1983); n) Bogdanova et al.: J Exp Biol **201**, 693 – 700 (1998); o) Garcia-Soto & Grinstein: Am J Physiol **258**, C1108 – C1116 (1990); p) Nauntofte & Dissing: Proc Finn Dent Soc **85**, 307 – 317 (1989); q) Maglova et al.: Am J Physiol **275**, C1330 – C1341 (1998); r) Garcia et al.: FEBS Lett **400**, 113 – 118 (1997).

measured in cultured rat aorta cells using a Cl^--sensitive fluorescent dye (MQAE) (31.6 mM) (9). The calculated reversal potentials for Cl^- were −29 and −40 mV, respectively, in these two preparations. These values of the reversal potential are marginal in terms of ability to activate voltage-dependent Ca^{2+} channels; however, a comparison of the contractions and depolarizations (achieved by increase in the extracellular K^+ concentration) shows that a depolarization to −40 mV is sufficient to produce contraction in smooth muscle cells (1). Extracellular Ca^{2+} is thought to be the main source of Ca^{2+} for smooth muscle contraction, especially for the tonic component, and activation of Cl^- channels and changes in $[Cl^-]_i$ are important because they regulate the activity of L-type Ca^{2+} channels.

Cl^- currents recorded in vascular cells

Two major Cl^- currents have been described in vascular smooth muscle cells, namely, Ca^{2+}-dependent Cl^- and volume-regulated Cl^- currents. The channel molecules responsible for the latter Cl^- current may be the ClC-3 isoform (ref. 10; also see review, ref. 11). The volume-regulated Cl^- channel can be activated experimentally by low osmotic pressure (perfusion with a hypotonic solution) or by certain pathological conditions. This Cl^- channel has also been reported to be activated by mechanical stress, which occurs during vascular distension caused by a rise in systolic pressure, and by urinary retention in the bladder etc. In these cases, a shift in the cellular responses toward contraction clearly favors both the maintenance of tissue integnity and systemic stability against mechanical stretch. Nelson et al. (12) demonstrated that raising the pressure in the cerebral artery to 80 mmHg depolarized the membrane, while Cl^--channel blockers, IAA-94 (indaryloxyacetic acid) and DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid), hyperpolarized the membrane and relaxed the tissue. These results indicated that stretching the vessel wall by increasing blood pressure activated the volume-regulated Cl^- channels and reduced the arterial diameter. The same group recently showed that the above Cl^--channel blockers also suppressed the non-selective cationic current (13). Thus, the importance of Cl^- channels to the pressure-induced response should promote further study and discussion, and development of selective Cl^--channel blockers would be an important aid to such investigations.

By use of the patch-clamp technique, Ca^{2+}-dependent Cl^- currents have been identified in various smooth muscle tissues, including vascular ones. It is of interest that it was the perforated patch-clamp technique (involving the use of nystatin or other antibiotics), rather than the conventional whole-cell voltage-clamp technique, that revealed the Ca^{2+}-dependent Cl^- current following agonist stimulation (14). This may reflect differences in the Ca^{2+}-buffering system between the two techniques. In case of the conventional whole-cell voltage-clamp technique, a strong Ca^{2+} chelater was always present in the pipette solution to maintain a low Ca^{2+} concentration within the cell ($[Ca^{2+}]_i$), whereas $[Ca^{2+}]_i$ is regulated by its own biological system in the perforated patch-clamp tehnique. Thus, the perforated patch-clamp technique keeps conditions more physiological, and quasi-physiological responses may therefore be seen in the cells under study. There are various pieces of evidence to suggest that the agonist-induced inward current is Ca^{2+}-dependent: i) low concentration of niflumic acid and stilbene derivatives partly inhibited the norepinephrine-induced inward current in the rabbit portal vein (15), while high concentrations completely blocked the histamine-induced inward current in the rabbit basilar artery (16); ii) removal of extracellular Ca^{2+} ultimately inhibited the agonist-induced inward current; and iii) the reversal potential of the agonist-induced inward current depended upon $[Cl^-]_i$ and $[Cl^-]_o$, but not on $[Na^+]_o$ and $[K^+]_o$ concentration. Furthermore, a redution in the amplitude of the agonist-induced current occurred following pretreatment with caffeine, CPA (cyclopiazonic acid) or thapsigargin. Thus, Ca^{2+} release from the caffeine-sensitive intracellular Ca^{2+} store sites and Ca^{2+} influx through L-type Ca^{2+} channels would seem to be important for activation of the Ca^{2+}-dependent Cl^- channels in these preparations. The Ca^{2+}-dependent Cl^- current is mainly activated by receptor stimulation in vascular cells; however, in some cases, Cl^- currents have been reported to be spontaneously activated. As spontaneous Cl^- currents were synchronously generated by the spontaneously Ca^{2+}-dependent K^+ currents (17, 18), this Cl^- current was thought to be activated by Ca^{2+} release from the store sites.

The anion permeability of Ca^{2+}-dependent Cl^- channels has been widely tested (see review, ref. 17). The order of anion permeability for Ca^{2+}-dependent Cl^- channels is $SCN^- > I^- > NO_3^- > Br^- > Cl^- \gg$ glutamate, which is a similar to the reported order for the volume-regulated Cl^- current in vascular cells and for the ClC-3 channel ($I^- > Br^- > Cl^-$) (11, 19). This order of anion permeability differs from those reported for other types of Cl^- channels such as ClC-0, ClC-1, ClC-2 and ClC-5 channels. The two types of Cl^- current (Ca^{2+}-dependent and volume-regulated Cl^- channels) can be separated by the use of certain channel blockers. Niflumic acid is one such blocker, and this agent inhibits Ca^{2+}-dependent Cl^- channels, but not volume-regulated Cl^- channels. DIDS, a stilbene derivative, is also known to inhibit the Ca^{2+}-dependent Cl^- channel rather than the volume-regulated channel.

Contribution of Cl^- conductance to smooth muscle contraction

To assess the physiological role played by Cl^- conductance in vascular smooth muscle cells, Criddle et al. (20, 21) examined the effects of niflumic acid on both K^+- and

agonist-induced contractions. They showed that niflumic acid (1 – 30 μM) selectively inhibited the agonist-induced contraction without changing the high K^+-induced contraction in both the rat aorta and rat mesenteric artery. Niflumic acid (30 μM), but not mefenamic acid (100 μM), was later shown by our group to reduce the amplitude of the histamine-induced contraction in the rabbit basilar artery without inhibiting the high K^+-induced contraction (22). This niflumic acid-induced inhibition was predominantly observed in the early phase of the agonist-induced contraction. Although a high concentration of niflumic acid blocks the high K^+-induced contraction, the contribution made by the Ca^{2+}-dependent Cl^- conductance to the contraction could be measured by carefully choosing the concentration of niflumic acid used. Criddle et al. (20) thought that the inhibitory action of niflumic acid on the agonist-induced contraction targeted Ca^{2+} influx through L-type Ca^{2+} channels since niflumic acid reduced neither the amplitude of the phasic contraction observed in Ca^{2+}-free solution (17) nor the Ca^{2+}-dependent K^+ current. On the other hand, Hogg et al. (15) thought that niflumic acid might act on the Ca^{2+} release mechanism on the basis of their observations. In our study (ref. 23, rabbit basilar artery), since a long exposure to niflumic acid (1 h or longer) led to the amplitude of the phasic contraction being reduced, a niflumic-acid-sensitive mechanism might contribute to an initial increase in the intracellular Ca^{2+} concentration through Ca^{2+} release from the intracellular Ca^{2+} store sites and resulted in contraction.

There have been several reports of the effects on smooth muscle contraction resulting from the replacement of extracellular Cl^- ions by other permeable or impermeable anions. Saha et al. (24) showed that use of Cl^--deficient solution (Cl^- substituted by glucronate and gluconate) reduced the muscle tone of the lower esophageal sphincter muscle of the opposum without changing the acetylcholine-induced contraction. This relaxation could be mimicked by application of the Cl^--channel blockers, NPPB (5-nitro-2-(3-phenylpropylamino)benzoic acid) and IAA-94, suggesting an important contribution of Cl^- conductance to esophageal muscle tone in the opposum. In rabbit basilar artery, use of low Cl^- solution (Cl^- substituted by several impermeable anions) reduced the amplitude of the histamine-induced contraction without changing the high K^+-induced contraction (23). In this preparation, histamine is a strong constrictor through activation of the H_1-receptor (16), and it induces a phasic contraction followed by a tonic contraction. The former depends on Ca^{2+} release from the store sites and the latter, on Ca^{2+} influx through L-type Ca^{2+} channels (Fig. 1). The typical effects produced by the use of low Cl^- solution on the histamine-induced contraction in the rabbit basilar artery are i) suppression of the phasic contraction, ii) suppression of the rate of rise of the tonic contraction and iii) increase in the rate of relaxation (Fig. 1). Thus, Cl^- conductance contributes to the enhancement of both the Ca^{2+}-induced Ca^{2+} release mechanism and Ca^{2+} influx. The rank order of potencies for the inhibitory effects of impermeable anions on the contraction resembled the rank order of permeabilities for the Ca^{2+}-dependent Cl^- channels in rat lacrimal gland cells (25).

Any direct evidence in favor of a contribution by Cl^- conductance in the plasmalemma to Ca^{2+} release from the sarcoplasmic reticulum (SR) is scarce, but few papers showed mutual relationships between Cl^- conductance on the SR membrane and the Ca^{2+} release from the ryanodine receptors. Using organic anions, Fruen et al. (26) demonstrated that substitution of Cl^- by propionate reduced Ca^{2+} release via a reduction in the functional rynodine receptor in skeletal muscle SR, but not in cardiac muscle SR. Thus, it is not clear whether and how Cl^- conductance on the SR mambrane participates in Ca^{2+} release from the SR. The difference observed between skeletal and cardiac muscles may due to the known presence of different ryanodine recepters in the two muscles, skeletal muscle possessing RYR-1 and RYR-3 receptors, whereas cardiac muscle possesses the RYR-2 receptor. All three types of RYR receptors may contribute to the Ca^{2+}-induced Ca^{2+} release

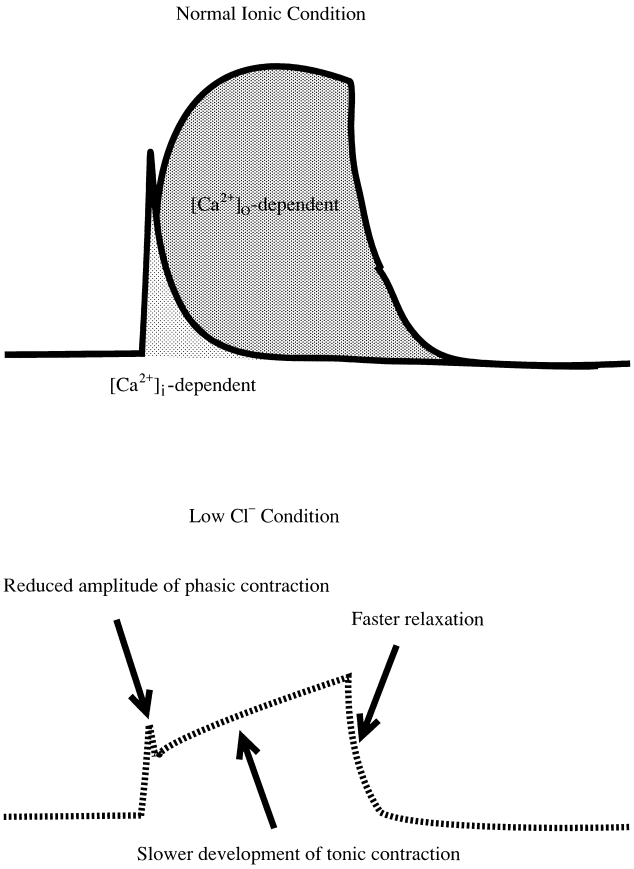

Fig. 1. Agonist-induced arterial contraction and its $[Cl^-]_o$-dependency.

mechanism in these cells, although it was reliably reported that Cl^--dependent activation of Ca^{2+} release occurs through the RYR-1 receptor in skeletal muscle (27). In the case of smooth muscle cells, it has been reported that RYR-2 and RYR-3 receptor genes are both present. If we assume that the RYR-2 receptor does not mediate Cl^--dependent Ca^{2+} release in smooth muscle cells, as it is reported to do in cardiac RYR-2 (27), then the RYR-3 receptor may mediate Ca^{2+} release from the SR in smooth muscle cells. As replacement of Cl^- with methanesulfonate reduced the Ca^{2+}-induced Ca^{2+} release in the skeletal muscle of RYR-1 knock-out mice (27), both RYR-1 and RYR-3 receptors seem to be regulated by the intracellular Cl^- concentration in skeletal muscle. Pollock et al. (14) reported no inhibition of Ca^{2+} uptake by niflumic acid in a rabbit stomach smooth muscle preparation, although several other Cl^--channel blockers did have an inhibitory action.

When $[Cl^-]_o$ was reduced to 60 mM (Cl^- substituted by glutamate), an increase in pressure in the lumen augmented the myogenic tone in the cerebral artery (28). The authors concluded that this might be caused by an increase in $[Ca^{2+}]_i$ through activation of the L-type Ca^{2+} channels, as the membrane was depolarized by a reduction in $[Cl^-]_o$ through a positive shift in the Cl^- reversal potential. Indeed, in the aorta when Cl^- was replaced by the permeable anion, SCN, there was a transient decrease in $[Ca^{2+}]_i$ followed the hyperpolarization of the membrane, and then $[Ca^{2+}]_i$ gradually increased within 20 min (28). When pressure was raised within the mesenteric artery, norepinephrine and 5-hydroxytryptamine both induced a contraction closely related to the Ca^{2+}-dependent Cl^- current without a change in the resting myogenic tone, whereas in cerebral artery stretched by strong pressure, a volume-regulated Cl^- current was activated under resting conditions (12, 21). As the former contraction was inhibited by niflumic acid, but not the latter, different Cl^- currents would appear to have been activated by the above maneuvers.

Contribution of Cl^- conductance to the membrane potential

Although much electrophysiological evidence of the presence of a Cl^- current and of its contribution to agonist-induced responses has been obtained in patch-clamp experiments, very few papers have been published in which microelectrode methods have been used. In anococcygeous muscle cells, Byrne and Large (29) reported that norepinephrine produced a membrane depolarization, the initial component of which was sensitive to $[Cl^-]_o$ (as substitution of Cl^- by the impermeable anion benzensulfonate abolished the initial membrane depolarization induced by norepinephrine). In these cells, although low Cl^- conditions inhibited the initial phase of the norepinephrine-induced contraction, its maximum amplitude remained unaltered, a result in good agreement with the effect of such conditions on the membrane potential (30). Recently, Gokina and Bevan (31) showed that niflumic acid, as well as DIDS, inhibited the membrane depolarization induced by histamine in the rabbit middle cerebral artery. Although a short application of niflumic acid did not produce a distinct repolarization on the histamine-induced depolarization in the rabbit basilar artery, a long pretreatment with niflumic acid could inhibit the depolarization (23). Similarly, superfusion with low-Cl^- solution (Cl^- substituted by impermeable anions) inhibited the histamine-induced depolarization (23). All these results indicate that part of the depolarization evoked by agonists was mediated by an activation of Ca^{2+}-dependent Cl^- conductance.

Summary

Taken together, the above reports demonstrate a contribution of Cl^- channels to agonist-induced contractions in vascular and other smooth muscle cells (Table 2). As blocker of Ca^{2+}-dependent Cl^- channels inhibited the early depolarization in the anococcygeous muscle, it would seem that the contraction that follows Ca^{2+} release by agonists is

Table 2. Roles of Cl^- channels in smooth muscle cells

Type	Gene	Supposed roles of Cl^- channels	Blockers or conditions (their actions)	References
Ca^{2+}-dependent Cl^- channels	?	contraction (early phase)	niflumic acid (relaxation)	20, 21, 22
		tonic contraction	gluconate substitution (relaxation)	24
			NPPB & IAA-94 (relaxation)	24
		contraction (early phase)	impearmeable anions (relaxation)	23, 30
		depolarization	impearmeable anions (repolarization)	29
		depolarization	niflumic acid & DIDS (repolarization)	23, 31
Volume-regulated Cl^- channels	ClC-3	osmotic homeostasis	IAA-94 & DIDS (hyperpolarization & relaxation)	12
			niflumic acid (no effect on membrane potential and contraction)	12
Cl^- channels in SR membrane		osmotic homeostasis	niflumic acid (no effect on Ca^{2+} uptake)	14

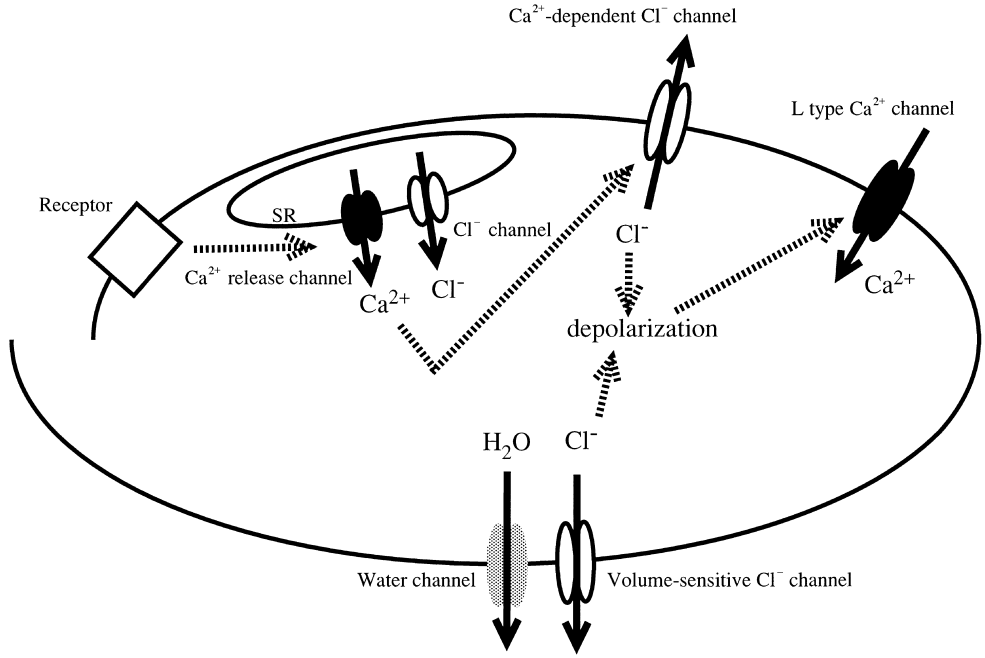

Fig. 2. Role of Cl^- channels in vascular smooth muscle cell.

directly regulated by Cl^- membrane conductance. Results from patch-clamp experiments have shown that niflumic acid (<50 μM) does not modify Ca^{2+} release by agonists and caffeine, as the Ca^{2+}-dependent K^+ current was not inhibited by their blocker (32). There is good evidence that the agonist-induced inward current can be attributed to Ca^{2+} release from the SR (pretreatment with caffeine or superfusion with Ca^{2+}-free solution inhibited the agonist-induced Cl^- current), and that an increase in $[Ca^{2+}]_i$ via the SR is essential for activation of Ca^{2+}-dependent Cl^- channels. On the other hand, a block of L-type Ca^{2+} channels by Ca^{2+} antagonists did not greatly change the agonist-induced depolarization of the membrane. Thus, a conclusion that Cl^--dependent membrane depolarization may regulate L-type Ca^{2+}-channel activation seems to be appropriate (Fig. 2). It is uncertain why Ca^{2+}-dependent Cl^- channels are opened by an increase in $[Ca^{2+}]_i$ caused by Ca^{2+} release from the store sites, but not by an increase in $[Ca^{2+}]_i$ caused directly by Ca^{2+} influx through L-type Ca^{2+} channels. In hair cells, Issa and Hudspeth (33) demonstrated a clustering and colocalization of Ca^{2+} channels and Ca^{2+}-dependent K^+ channels in the presynaptic active zone. Although no evidence that clustering also occurs in smooth muscle has so far been published, it could be that Ca^{2+}-dependent Cl^- channels are more densely distributed close to the Ca^{2+} store sites, so that activation of these channels can easily occur following a $[Ca^{2+}]_i$ increase through Ca^{2+} release. One other possible reason, for there being distinct contributions by store sites and L-type Ca^{2+} channels to the activation of Ca^{2+}-dependent Cl^- channels, include the predominant contribution to tonic contraction being made by agonist-induced nonselective cation channels.

To assess the physiological and pathophysiological roles played by Cl^- channels, a more specific channel blocker is required, as the present channel blockers, such as niflumic acid and stilbene derivatives, are known to act at various sites. Furthermore, replacement of Cl^- by other organic and inorganic anions changes the cellular conditions such as intracellular pH and cell volume through a suppression of Cl^--sensitive exchangers and other Cl^- channels. Indeed, experiments with low Cl^- solution have produced a wide variety of effects in anococcygeous and vascular smooth muscle cells (23, 29). The results of such experiments might depend upon whether the substitutes do or do not have an action similar to that of Cl^-, which will be strongly related to their permeability through the cell and SR membranes.

REFERENCES

1 Casteels R, Kitamura K, Kuriyama H and Suzuki H: The membrane properties of the smooth muscle cells of the rabbit main pulmonary artery. J Physiol (Lond) **271**, 41 – 61 (1977)
2 Aickin CC and Brading AF: Measurement of intracellular chloride in guinea-pig vas deferens by ion analysis, ^{36}Cl efflux and microelectrodes. J Physiol (Lond) **326**, 139 – 154 (1982)
3 Brading AF: Analysis of the effluxes of sodium, potassium and chloride ions from smooth muscle in normal and hypertonic solutions. J Physiol (Lond) **214**, 393 – 416 (1971)

4 Aickin CC and Vermue NA: Microelectrode measurement of intracellular chloride activity in smooth muscle cells of guinea-pig ureter. Pflugers Arch **397**, 25 – 28 (1983)

5 Chipperfield AR, Davis JP and Harper AA: Sodium-independent inward chloride pumping in rat cardiac ventricular cells. Am J Physiol **272**, H735 – H739 (1997)

6 Lai ZF and Nishi K: Intracellular chloride activity increases in guinea-pig ventricular muscle during stimulated ischemia. Am J Physiol **275**, H1613 – H1619 (1998)

7 Piwnica-Worms D, Jacob R, Horres CR and Lieberman N: Transmembrane chloride flux in tissue-cultured chick heart cells. J Gen Physiol **81**, 731 – 748 (1983)

8 Fong CN and Hinke JA: Intracellular Cl activity, Cl binding, and ^{36}Cl efflux in rabbit papillary muscle. Can J Physiol Pharmacol **59**, 479 – 484 (1981)

9 Koncz C and Daugirdas JT: Use of MQAE for measurement of intracellular[Cl^-] in cultured aortic smooth muscle cells. Am J Physiol **267**, H2114 – H2123 (1994)

10 Yamazaki J, Duan D, Janiak R, Kuenzli K, Horowitz B and Hume JR: Functional and molecular expression of volume-regulated chloride channels in canine vascular smooth muscle cells. J Physiol (Lond) **507**, 729 – 736 (1998)

11 Yamazaki J and Kitamura K: Ion channels in vascular smooth muscle. *In* Heart Physiology and Pathophysiology, 4th Ed, Edited by Sperelakis N, Kurachi Y, Terzic A and Cohen MV, pp 327 – 355, Academic Press, San Diego (2000)

12 Nelson MT, Conway MA, Knot HJ and Brayden JE: Chloride channel blockers inhibit myogenic tone in rat cerebral arteries. J Physiol (Lond) **502**, 259 – 264 (1997)

13 Welsh DG, Nelson MT, Eckman DM and Brayden JE: Swelling-activated cation channels mediate depolarization of rat cerebrovascular smooth muscle by hyposmolarity and intravascular pressure. J Physiol (Lond) **527**, 139 – 148 (2000)

14 Pollock NS, Kargacin ME and Kargacin GJ: Chloride channel blockers inhibit Ca^{2+} uptake by the smooth muscle sarcoplasmic reticulum. Biophys J **75**, 1759 – 1766 (1998)

15 Hogg RC, Wang Q and Large WA: Action of niflumic acid on evoked and spontaneous calcium-activated chloride and potassium currents in smooth muscle cells from rabbit portal vein. Br J Pharmacol **112**, 977 – 984 (1994)

16 Kamouchi M, Ogata R, Fujishima M, Ito Y and Kitamura K: Membrane currents evoked by histamine in rabbit basilar artery. Am J Physiol **272**, H638 – H647 (1997)

17 Large WA and Wang Q: Characteristics and physiological role of the Ca^{2+}-activated Cl^- cnductance in smooth muscle. Am J Physiol **271**, C435 – C454 (1996)

18 Kamouchi M, Fujishima M, Ito Y and Kitamura K: Simultaneous activation of Ca^{2+}-dependent K^+ and Cl^- currents by various forms of stimulation in the membrane of smooth muscle cells from the rabbit bailar artery. J Smooth Muscle Res **34**, 57 – 68 (1998)

19 Greenwood IA and Large WA: Properties of a Cl^- current activated by cell swelling in rabbit portal vein vascular smooth muscle cells. Am J Physiol **275**, H1524 – H1532 (1998)

20 Criddle DN, Soares de Moura R, Greenwood IA and Large WA: Effects of niflumic acid on noradrenaline-induced contractions of the rat aorta. Br J Pharmacol **118**, 1065 – 1071 (1996)

21 Criddle DN, Soares de Moura R, Greenwood IA and Large WA: Inhibitory action of niflumic acid on noradrenaline- and 5-hydroxytryptamine-induced pressor responses in the isolated mesenteric vascular bed of the rat. Br J Pharmacol **120**, 813 – 818 (1996)

22 Uchida R, Nishijima H, Yamazaki J, Shimamura K and Kitamura K: Effects of Cl channel blockers on the contractions recorded in rabbit basilar artery. Jpn J Pharmacol **79**, Suppl **I**, 210P (1990)

23 Uchida R, Nishijima H, Yamazaki J, Shimamura K, Kato K, Ohkubo T and Kitamura K: Inhibition of histamine-induced contraction during a long exposure to low $[Cl]_o$ condition of the rabbit basilar artery. Jpn J Pharmacol **82**, Suppl **I**, 208P (2000)

24 Saha JK, Sengupta JN and Goyal RK: Role of chloride ions in lower esophageal sphincter tone and relaxation. Am J Physiol **263**, G115 – G126 (1992)

25 Evans MG and Marty A: Calcium-dependent chloride currents in isolated cells from rat lacrimal glands. J Physiol (Lond) **378**, 437 – 460 (1986)

26 Fruen BR, Kane PK, Mickelson JR and Louis CF: Chloride-dependent sarcoplasmic reticulum Ca^{2+} release correlates with increased Ca^{2+} activation of ryanodine receptors. Biophys J **71**, 2522 – 2530 (1996)

27 Ikemoto T, Komazaki S, Takeshima H, Nishi M, Noda T, Iino M and Endo M: Functional and morphorogical features of skeletal muscle from mutant mice lacking both type 1 and type 3 ryanodine receptors. J Physiol (Lond) **501**, 305 – 312 (1997)

28 Standley PR, Zhang F, Ravi J, Ram JL and Sowers JR: Effects of SCN substitution for Cl^- on tension, [Ca^{2+}], and ionic currents in vascular smooth muscle. Life Sci **59**, 739 – 752 (1996)

29 Byrne NG and Large WA: Evidence for two mechanisms of depolarization associated with a1-adrenoceptor activation in the rat anococcygeous muscle. Br J Pharmacol **86**, 711 – 721 (1995)

30 Large WA: The effect of chloride removal on the responses of the isolated rat anococcygeus muscle to alpha1-adrenoceptor stimulation. J Physiol (Lond) **352**, 17 – 29 (1994)

31 Gokina NI and Bevan JA: Role of intracellular Ca^{2+} release in histamine-induced depolarization in rabbit middle cerebral artery. Am J Physiol **278**, H2105 – H2114 (2000)

32 Hogg RC, Wang Q and Large WA: Effects of Cl channel blockers on Ca-activated chloride and potassium currents in smooth muscle cells from rabit portal vein. Br J Pharmacol **111**, 1333 – 1341 (1994)

33 Issa NP and Hudspeth AJ: Clustering of Ca^{2+} channels and Ca^{2+}-activated K^+ channels at fluorescently labeled presynaptic active zones of hair cells. Proc Natl Acad Sci USA **91**, 7578 – 7582 (1994)