Ann. Rev. Pharmacol. Toxicol. 1983. 23:373–96

MECHANISMS OF CALCIUM ANTAGONIST-INDUCED VASODILATION

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INTRODUCTION

Excitation-contraction coupling in smooth and cardiac muscle can be disrupted by a number of organic compounds known collectively as Ca antagonists (CAts). In 1964, Fleckenstein (1) provided the first important clue regarding their possible mechanism of action when he noted that the cardiac effects of two of these agents, verapamil and prenylamine, were similar to those of Ca^{2+} removal from the extracellular space. A few years later, he proposed that CAts exerted their negative inotropic and coronary vasodilator effects by blocking the entry of Ca^{2+} into the cells (2, 3). The vasodilator and cardiac effects of CAts are having a great impact on cardiovascular medicine; consequently many reviews and monographs on this subject have appeared in the recent past (4–9). The purpose of this review is to provide an in-depth analysis of the mechanisms of action of CAts, rather than to compile an exhaustive survey of the literature. Only when we know how CAts interact with the processes that regulate Ca availability and sensitivity in smooth muscle can we understand why agents that "antagonize" the action of such a universal intracellular messenger can be effectively deployed as selective therapeutic agents in cardiovascular medicine.

MECHANISM OF ACTION OF CA ANTAGONISTS

Inhibition of Ca Influx

In 1969, Godfraind & Kaba (10) found that cinnarizine preferentially blocked the slow (S) phase of the rat aortic contraction induced by norepinephrine (Ne). Bohr (11) had previously shown that the magnitude of the

S phase depended directly on the extracellular Ca concentration ($[Ca]_e$), while the fast transient (F) phase of the epinephrine contraction demonstrated an inverse relationship with respect to $[Ca]_e$. Although these early observations set the stage for our present understanding of the principal mechanism of action of CAts, there still remained considerable confusion regarding the Ca sources for smooth muscle contraction.

Hinke (12) carefully examined the dependencies on $[Ca]_e$ of rat caudal artery contractions due to potassium depolarization and Ne. He found the Ne contractions to be much more resistant to Ca removal then the K contractions and concluded that there were two extracellularly bound Ca fractions: a "loosely bound" one activated by K, and a "tightly bound" one sensitive to Ne. Daniel (13) and Hudgins & Weiss (14) suggested that release of intracellular Ca may also be involved in agonist-induced contractions.

The discovery by Lettvin et al (15) that the trivalent cation lanthanum can be used to displace Ca^{2+} from biologically important sites proved essential to the clarification of the above problems. In the rabbit aorta, La^{3+} completely abolished the K contraction and the S phase of the Ne contraction, but left the Ne-induced F phase intact (16–18). Since La^{3+} displaces extracellularly bound Ca (19) and inhibits Ca transport across biological membranes (20, 21), it was established that high K depolarization and Ne mobilize extracellular Ca, while Ne in addition releases Ca from an intracellular store. The use of La^{3+} to quench extracellularly bound Ca in order to measure the smaller amount of intracellular Ca also made it possible to determine the direct effects of CAts on ^{45}Ca uptake into smooth muscle cells (17). This determination is critical in elucidating the mechanism of action of CAts for the following reasons: 1. since smooth muscle contraction can be initiated from both intra- and extracellular Ca sources, depression of tension alone does not provide the exact site of inhibition; and 2. a parallel inhibition of contraction and ^{45}Ca influx, in the case of contractions that are entirely dependent on extracellular Ca, would provide strong evidence for an exclusive inhibition of Ca entry. The validity of the latter statement is based on the assumption that inhibitory actions on the contractile proteins or Ca pumps would tend to disrupt the close correlation between Ca influx and contractile tension.

Mayer et al (22), the first to apply such Ca flux measurements to the study of CAts, showed that D600 inhibited the net ^{45}Ca uptake, which was associated with high K depolarization or with spontaneous activity in the guinea pig taenia coli. The ^{45}Ca uptake that occurred in quiescent smooth muscle was not blocked by D600. Rosenberger et al (23) extended these observations to the dihydropyridine BAY 1040 inhibition of ^{45}Ca uptake induced by cholinergic activation. However, in order to provide convincing

evidence for the thesis that CAts inhibit smooth muscle contraction by blocking stimulated Ca influx, it is not enough to find a close correlation between relaxation and depression of net ^{45}Ca uptake, as the latter can be complicated by changes in ^{45}Ca efflux. However, when inhibition of high K-stimulated unidirectional ^{45}Ca influx (measured over time periods of 1 to 2 min) was compared to inhibition of high K contractions, a very close correlation was also obtained (24–26). These results establish the mechanism of action of the CAts so tested (nisoldipine, diltiazem, D600, and flunarizine) to be exclusively blockade of stimulated Ca influx, since other actions (e.g. on calmodulin, Ca release, or Ca efflux) would disrupt the above close correlation.

A general property of organic CAts is that they are able to block stimulated ^{45}Ca uptake but do not significantly affect resting Ca influx (23–26). In this respect, CAts differ sharply from La^{3+}, which blocks all Ca entry into cells, including that entering through the passive membrane leak pathway (19). Another difference between La^{3+} and CAts is that the former will inhibit ionophore-mediated Ca^{2+} flux (27), whereas the latter will not (28). The actions of the trivalent cation La^{3+} can be explained by its competition with Ca^{2+} for negatively charged binding sites. Such sites are probably involved in all Ca^{2+} transport processes across the cell membrane. In contrast, CAts are generally organic noncharged lipophilic molecules of widely varying structures, which bear little resemblance to calcium ions in either structure or charge density. For this reason it was somewhat surprising that reversible competition was described between CAts and Ca^{2+} (29–31). However, a recent report shows that at somewhat higher CAt concentrations $[Ca]_e$ is no longer able to overcome the inhibition completely (24). Our present view is that CAts do not reversibly compete with Ca^{2+} for binding to Ca transport sites, but that CAts specifically bind to various components of excitable Ca^{2+} channels causing their inactivation. At concentrations that generally exceed therapeutic levels, CAts do exert additional effects, as discussed below.

Inhibition of Intracellular Ca Release

The early studies of Godfraind & Kaba (10), Kalsner et al (32), Peiper et al (33), and Deth & Van Breemen (18) all indicate that the initial (F) phase of the Ne contraction, which is due to intracellular Ca release, is rather insensitive to inhibition by CAts. Typically, preapplication of a dose of CAt just large enough to block stimulated Ca influx will cause the Ne contraction to become transient and then plateau at a level somewhere between the peak and resting tension levels. Since the plateau of the Ne contraction is due to stimulated Ca influx, it was surprising that a diminished contracture should persist, in spite of complete CAt-mediated inhibition of

Ne-stimulated Ca influx. Loutzenhiser & Van Breemen (26) have recently proposed the following explanation for this apparent paradox. They found that the agonist-releasable intracellular Ca store (ARC) sequesters Ca near the inner surface of the cell membrane. This Ca buffering action of the ARC prevents any tension development that might otherwise result from the passive Ca leak, which is not blocked by CAts. However, when Ne or another agonist releases Ca from the ARC, its cytoplasmic Ca buffering action is abolished, causing the Ca leak to become effective in tension development. Thus, even if the stimulated Ca influx is blocked, tension will not return to resting levels as long as the agonist is present.

Recently, Church & Zsoter (34) and Walus et al (35) suggested that all or part of CAt-induced vasodilation may be due to inhibition of Ca release. The latter group found that Ne-induced contractions of canine mesenteric arterial strips were equally sensitive to nifedipine, whether they were exposed to 1.17 mM Ca or Ca-free solution. Saida & Van Breemen (36) have compared the sensitivities to diltiazem and nisoldipine of Ne contractions of thin bundles of rabbit mesenteric artery under control (1.5 mM Ca) and Ca-free (0 Ca, 2 mM EGTA) conditions. They found that diltiazem did inhibit intracellular Ca release, but only at concentrations exceeding 10^{-4} M. High concentrations of nisoldipine ($> 10^{-5}$ M) could only slightly inhibit the Ca-free contractions. Another measure of intracellular Ca release can be obtained by the addition of caffeine to mildly saponin-skinned smooth muscle (37, 38). Such a preparation has a plasmalemma that is very leaky to Ca^{2+} while intracellular membranes remain intact. In this preparation the caffeine contractions result entirely from Ca release from sarcoplasmic reticulum (SR). Saida & Van Breemen (39) found the same low sensitivity of this caffeine-induced skinned mesenteric artery contraction to diltiazem as was described for the Ca-free Ne contractions. These results led to the conclusion that intracellular Ca release can be inhibited to varying degrees by certain CAts, but that this effect does not appear to play a role at therapeutic concentrations.

Stimulation of Ca Extrusion

Several groups have postulated that some CAts exert a relaxing effect due to stimulation of Ca efflux. The smooth muscle membrane has two Ca extrusion mechanisms, a Ca ATPase (40, 45) and a Na,Ca exchanger (41). Numerous experiments on intact tissues have indicated that the Ca ATPase plays a major role in Ca extrusion and homeostasis, whereas the Na,Ca exchanger is of minor importance (42–44). This idea has recently been corroborated by the finding that in isolated plasmalemma the maximum Ca transport rate of the Ca ATPase is about one order of magnitude greater than that of the Na,Ca exchanger [(45, 46), T. Godfraind, personal communication]. The latter also has a lower affinity for Ca^{2+}.

Although the mechanism is not clear, there does appear to be a correlation between Na content and vascular smooth muscle tone (47, 48). In this context, it is intriguing that several groups have suggested that CAts may stimulate the membrane Na,K pump. Flaim and coworkers (49, 50) reported a diltiazem-induced ouabain-sensitive stimulation of O_2 consumption coupled with a decrease in cellular ^{22}Na. He thus concluded that diltiazem caused relaxation by stimulating Na-Ca exchange through an elevated Na gradient (51). Mikkelsen et al (52) have also found a nifedipine-induced decrease in cell ^{22}Na.

Pan & Janis (53) recently demonstrated that nimodipine but not diltiazem stimulated the Na,K ATPase of smooth muscle microsomes. When ion fluxes in intact smooth muscle were examined, however, no stimulation of the Na,K pump could be shown (R. Deth, personal communication). More studies are awaited before the effects of CAts on Na fluxes are clarified. However, at this point it is not at all clear how changes in cellular [Na], if they are caused by CAts in intact vascular smooth muscle, could prevent contractions induced by depolarization and agonists. Furthermore, ^{45}Ca flux studies have clearly demonstrated CAt-induced vasodilation to be a function of inhibition of ^{45}Ca influx but not of stimulation of ^{45}Ca efflux. ^{45}Ca efflux during rest and Ne stimulation are not affected by flunarizine, verapamil, D600, or diltiazem (25, 34, 54–56). D600 inhibits K^+ stimulated ^{45}Ca efflux (54), but this effect is secondary to the D600 blockade of high K-stimulated influx of ^{40}Ca that would otherwise displace ^{45}Ca from intracellular sites (57). The only CAt reported to stimulate ^{45}Ca efflux is nifedipine (34). This stimulation was observed in nonstimulated arterial smooth muscle and has not been correlated with nifedipine effects on contractility. We conclude that the evidence described above does not at this time favor a role for Ca extrusion pumps in CAt-induced vasodilation.

Effects on Calmodulin

Boström et al (58) postulated that the dihydropyridine CAt felodipine caused relaxation by interacting with calmodulin. These investigators and Johnson et al (59) showed that at least some CAts, including felodipine, are capable of such interactions. Johnson et al (59) also demonstrated that prenylamine, verapamil, and diltiazem inhibited calmodulin activation of myosin light chain kinase, although the concentrations of verapamil and diltiazem needed for this effect were a thousand fold higher than those necessary for vasodilation. In order to determine if direct inhibition of myofilament activation is relevant to the pharmacologic action of diltiazem and nisoldipine, Saida & Van Breemen (36) compared their dose-response curves in intact mesenteric arterial strips contracted with K to those obtained in the same strips stimulated with Ca after "saponin skinning." The K contractions were inhibited 50% at 4×10^{-7} M diltiazem and 2×10^{-9}

M nisoldipine. The Ca-induced contractions of the skinned fibers were inhibited 50% by 2 X 10^{-4} M diltiazem, and not significantly at all by nisoldipine. Rüegg (personal communication) has also failed to observe relaxation of skinned (arterial) smooth muscle fibers by pharmacologically active concentrations of felodipine. The same laboratory has even found stimulation of Ca-activated contractions in skinned guinea pig taenia coli by the CAts verapamil and prenylamine (60).

This lack of correlation between inhibition of calmodulin function and vasodilation precludes calmodulin from being the pharmacologic receptor site for the CAts. Furthermore, the known therapeutic specificity of CAts argues against calmodulin being their receptor site, since Ca-calmodulin binding represents a late step in the chain of events leading to activation. The ubiquity of calmodulin would also be inconsistent with the specificity of CAt action.

CA ANTAGONIST BINDING TO MEMBRANES

Although the agents classified as CAts have widely varying chemical structures their great potency and selectivity indicate the existence of specific CAt receptors. The evidence presented above suggests that these sites are associated with excitable Ca channels in the smooth muscle plasmalemma. The chemical nature of these sites can be gleaned from structure-activity relationships determined for both pharmacological activity and ligand binding. For the former we refer the reader to a review by Triggle (see 61). In short, his conclusions are that for each different group of compounds, represented by the prototypes verapamil, diltiazem, and nifedipine, there are specific structural requirements for activity. These have been worked out in detail only for the dihydropyridines. Furthermore, all three categories exhibit stereoselectivity, which in the case of verapamil is only directed towards the Ca channels but not to its blockade of Na channels and adrenergic receptors. The fact that the same channels may be inhibited by compounds of rather different structures, which include the lanthanides, also suggests that Ca channels have more than one structural site at which inhibition can be effected.

The recent synthesis of radiolabeled nitrendipine has spurred great productivity in the study of CAt ligand binding. Bolger et al (62) described specific [^{3}H] nitrendipine binding to guinea pig ileal smooth muscle microsomes characterized by a K_D of .16 nM and a maximum binding capacity of 1.1 picomole per mg protein. Studies of structure-activity relationships showed all the dihydropyridines to act at the same site, with a good correlation existing between their potency in displacing [^{3}H] nitrendipine and pharmacological potency. The same stereoselectivity was found for binding

and inhibition of contraction. Several laboratories have also found that verapamil and D600 partially inhibit [^{3}H] nitrendipine binding, whereas diltiazem enhances it [(62, 63), D. J. Triggle, personal communication]. Although there are a number of possible explanations for this latter observation, it indicates that the binding sites for these three CAts are not identical and that they may interact allosterically (63-65). The kinetics for polyvalent cation interactions with the nitrendipine binding sites have not yet been completed, but preliminary reports show that both Ca^{2+} and La^{3+} are able to displace labeled nitrendipine (63, 65). Although these initial studies show great promise, some problems have also surfaced. For example [^{3}H] nitrendipine binds to cardiac sarcolemma with the same affinity as to smooth muscle membranes in spite of the fact that the latter is much more sensitive to its inhibitory actions.

It is anticipated that future binding studies with radioactively labeled CAts will yield information about the linkage of receptors to Ca channels. The initial results indicate that there are fourteen times as many muscarinic receptors as [^{3}H] nitrendipine binding sites in intestinal smooth muscle (62), and that there are 360 times as many [^{3}H] flunarizine binding sites in the rat aorta (63, 66) as [^{3}H] nitrendipine binding sites in the guinea pig ileal smooth muscle. As these studies become more sophisticated, we may eventually learn the chemical nature of Ca channels and their possible involvement in such vascular smooth muscle diseases as coronary vasospasm and hypertension.

SELECTIVITY OF CA ANTAGONISTS

The molecular basis of the selectivity exhibited by CAts resides in the ability of these agents to interact with specific Ca^{2+} channels in the plasmalemma as described above. The ability of CAts to block smooth muscle contraction selectively over neurotransmission or cardiac function implies that the Ca^{2+} entry pathways in smooth muscle differ qualitatively from those found in other tissues. Selectivity of CAts in different parts of the circulatory system appears to be related to differences in the Ca sources and types of Ca channels used to mediate Ca influx. It is well known that the initial phase of agonist-induced large artery contraction is due to intracellular Ca release, which is rather resistant to CAts. The intracellular Ca released during the initial phase, however, does not contribute to the tonic contractions; hence, selectivity here would depend on differences among vascular tissues in their pathways of stimulated Ca entry. We find that the tonic contractions of all vascular smooth muscle depend on the opening of at least two types of channels, potential-operated and receptor-operated. The different identities of these types of channels forms an obvious basis for specificity of CAt

action. We discuss below selectivity of CAts within a given vascular tissue, and among vascular tissues, and attempt to outline hypotheses that may provide theoretical bases for such selectivities.

Electrically Gated Ca Channels (POCs) vs Ca Pathways Regulated by Receptors (ROCs)

In 1979, two groups hypothesized that the Ca influx stimulated by high K depolarization and by Ne receptor occupation in vascular smooth muscle occurs through separate pathways (43, 67). The pathway activated by K depolarization was termed the potential-operated channel (POC), and that by receptor occupation the receptor-operated channel (ROC). There are basically three lines of evidence to support this hypothesis: 1. The tonic Ne contraction is dependent on extracellular Ca, but may occur in the absence of depolarization; 2. ^{45}Ca influx stimulated by high K depolarization and Ne is additive; and 3. CAts exert selective effects on one channel over the other.

A number of studies have indicated that Ne can induce smooth muscle contraction independently of membrane depolarization. We know that Ne can contract vascular smooth muscle that has already been depolarized (68). Droogmans et al (69) have shown that maximal Ca influx-dependent contraction of the rabbit ear artery with 10^{-6} M Ne occurred in the absence of any change in membrane potential. Casteels et al (70) have shown in the rabbit main pulmonary artery that Ne contractions induced by concentrations below 10^{-7} M were not associated with membrane depolarization. Higher Ne concentrations produced further tension and a maximal membrane depolarization of about 10 mV. K-induced contractions and membrane depolarization were, on the other hand, closely correlated for [K] from 2 to 100 mM. Haeusler (71) has also reported that Ne induced about a 10 mV depolarization in rabbit pulmonary artery, but it occurred at [Ne] between 10^{-8} and 10^{-6} M. Higher [Ne] produced a large increase in tension without further membrane depolarization. These studies all demonstrate that Ne-induced Ca influx can occur through a pathway that is not activated by membrane depolarization. Hermsmeyer et al (72), in contrast, have shown that membrane depolarization and tension are correlated for the entire dose range of Ne in the rat caudal artery. Ne-induced depolarization reported in this (72) and other studies (69, 79) may be secondary rather than causal to Ca influx, because it can be blocked in the rabbit pulmonary artery by diltiazem (73) and in the rat caudal artery by nitrendipine (K. Hermsmeyer, personal communication). On the other hand, diltiazem has little or no effect on high K-induced membrane depolarization or on resting membrane potential (73–75). These data suggest that CAts do not exert their relaxant effects through membrane hyperpolarization, and that high K

induces contraction by depolarizing the plasma membrane, thereby inducing Ca influx through a POC. Ne-induced contraction does not appear to be dependent on membrane depolarization.

Evidence supporting the concept of separate populations of channels activated by membrane depolarization and receptor occupation was provided by a study by Meisheri et al (76). They have shown that unidirectional ^{45}Ca influx stimulated by 80 mM K and 10^{-6} M Ne are additive when the two modes of activation are applied simultaneously to rabbit aorta. These results rule out the possibility that Ne and K activate the same channel but by differing mechanisms. The possibility exists, however, that Ne induces Ca influx through both the POC and the ROC, as Ca influx through the ROC could induce a secondary membrane depolarization.

The third line of evidence for separate POCs and ROCs is that ^{45}Ca influx induced by 80 mM K in rabbit aorta is inhibited completely by concentrations of D600, diltiazem, and nisoldipine that inhibit only 10–20% of that induced by 10^{-6} M Ne (24, 26, 76). These data indicate that the CAts are relatively selective for the POC over the ROC. Moreover, Ne cannot induce a substantial secondary membrane depolarization in this tissue, because Ca influx stimulated through the POC would show high sensitivity to the CAts, whereas that induced by Ne does not. Hence, it seems clear that POCs and ROCs of separate identity do exist. Furthermore, different ROCs may be activated by different agonists.

ARE POCs MORE SENSITIVE TO CA ANTAGONISTS THAN ROCs? The concept that the POC is more sensitive to CAts than the ROC is supported by the finding that in most vascular tissues K-induced contractions are more sensitive to CAts than Ne-induced contractions (see Table 1). In rabbit aorta, K-induced contractions have been shown to be more sensitive to inhibition by the CAts TMB-8 (77), nisoldipine (26, 78), D600 (76, 79), diltiazem (24), cinnarizine (80), and nifedipine (79) than Ne-induced contractions. Similar results have been obtained for D600 in rabbit mesenteric (79), basilar, and ear arteries (81); for diltiazem in the rabbit renal artery (82); for diltiazem and nifedipine in the rat portal vein (34) and in the perfused rat mesenteric vascular bed (83); for cinnarizine (80) and nisoldipine (36) in rabbit mesenteric artery; for flunarizine, cinnarizine, and nifedipine in rat aorta (84); and for nifedipine and flunarizine in rat mesenteric artery (84). Furthermore, contraction induced by $PGF_{2\alpha}$ in the human pial artery, and dog coronary and mesenteric arteries, is less sensitive to inhibition by nifedipine (85) and verapamil (86), respectively, than is activation by depolarization.

A more direct approach to the study of POC and ROC sensitivities is to measure CAt inhibition of ^{45}Ca influx induced by Ne- and K-depolariza-

tion. When this was done in rabbit aorta, diltiazem (24), D600 (76), and nisoldipine (26) were shown to be more potent in inhibiting ^{45}Ca influx induced by 80 mM K than that induced by 10^{-6} M Ne. It may be argued that the ROC sensitivity to CAts cannot be directly deduced from these results, since Ne-induced Ca influx through the ROC may cause secondary membrane depolarization and consequent POC activation. If this occurs, albeit to a small extent in this vessel, then the CAt sensitivity of the Ne-induced ^{45}Ca influx may partly reflect POC sensitivity. Hence, the ROC may be even less sensitive to CAts than the contraction and ^{45}Ca influx studies indicate.

In contrast, several studies indicate that the ROC is more sensitive to CAts than the POC in some tissues (see Table 1). Walus et al (35) have shown that K-contracted strips of isolated canine mesenteric arteries are less sensitive to nifedipine than are Ne-contracted strips. Rat superior mesenteric arterial strips contracted with a K depolarizing solution were shown to be about equisensitive to cinnarizine as those contracted with Ne (84). Bevan (82) has also found that in the rabbit basilar artery Ne-induced contractions are more sensitive to diltiazem than K-induced contractions, whereas they are about equisensitive in the ear and mesenteric arteries. Verapamil is about equipotent in inhibiting K- and Ne-induced contractions of the perfused mesenteric vascular bed of the rat (83), and in inhibiting $PGF_{2\alpha}$- and K-induced contractions of the dog cerebral artery (86). Moreover, Cauvin et al (87) have shown that Ne contractions of rabbit mesenteric resistance vessels are far more sensitive to diltiazem than K-induced contractions. Since these vessels do not utilize intracellular Ca release as a mechanism of contraction for either K or Ne activation, these results strongly indicate that the ROC itself is much more sensitive to diltiazem in the resistance vessels than the POC. Again, it is not known to what degree the agonists induce membrane depolarization in these vessels, so the sensitivity of agonist-induced contractions to CAts may again represent the combined sensitivities of ROC and POC. If so, then the CAt sensitivity of the ROC could be inferred to be even greater in these vessels than the contraction data suggest. In conclusion, the often-stated generalization that POCs are more sensitive than ROCs to CAts does not always apply.

Ca Antagonist Selectivity for Particular Vascular Beds

There have been numerous comparative studies describing greater sensitivity to CAts in certain vascular tissues than in others. In making such comparisons, the particular activating mechanism utilized to contract the tissues must be taken into account. It appears that the ROC may vary much more from tissue to tissue in its sensitivity to CAts than does the POC (see Table 1).

Table 1 IC_{50}s[a] for inhibition of contraction

CAt(M)	Vessels compared	Modes of activation		References
		NE	K	
Nifedipine	Rat aorta	2×10^{-8}	3×10^{-9}	84
	mesenteric artery	1×10^{-8}	4×10^{-9}	84
	mesenteric bed	$> 10^{-6}$	1×10^{-7}	83
	Dog mesenteric artery	8×10^{-8}	1×10^{-6}	35
		$PGF_{2\alpha}$		
	cerebral artery	3×10^{-8}	—	86
	mesenteric artery	5×10^{-7}	—	86
		NE		
	Rabbit aorta	3×10^{-5}	1×10^{-8}	79
	mesenteric artery	2×10^{-6}	1×10^{-8}	79
		$PGF_{2\alpha}$		
	Human pial artery	2×10^{-8}	7×10^{-9}	85
	omental artery	—	2×10^{-8}	85
		NE		
	Human mesenteric artery	2×10^{-6}	1×10^{-7}	52
	mesenteric vein	3×10^{-6}	1×10^{-7}	52
Cinnarazine	Rat aorta	2×10^{-7}	3×10^{-8}	84
	mesenteric artery	6×10^{-8}	9×10^{-8}	84
	caudal artery	—	4×10^{-7}	92
	Rabbit aorta	$>> 10^{-5}$	5×10^{-6}	80
	mesenteric artery	10^{-5}	3×10^{-8}	80
	ear artery	—	8×10^{-8}	92
		$PGF_{2\alpha}$		
Verapamil	Dog cerebral artery	2×10^{-7}	2×10^{-7}	86
	coronary artery	$> 10^{-5}$	3×10^{-7}	86
	mesenteric artery	$> 10^{-5}$	5×10^{-7}	86
		NE		
	Rat mesenteric bed	6×10^{-6}	6×10^{-6}	83
	Human mesenteric artery	3×10^{-6}	3×10^{-6}	52
	mesenteric vein	3×10^{-6}	4×10^{-6}	52
D600	Rabbit aorta	10^{-4}	4×10^{-8}	79
		6×10^{-6}	1×10^{-7}	76
	mesenteric artery	4×10^{-5}	5×10^{-8}	79
	basilar artery	6×10^{-8}	5×10^{-9}	81
	ear artery	6×10^{-7}	4×10^{-9}	81
	Dog coronary	1×10^{-6}	1×10^{-7}	89
		5HT		
	Rabbit basilar artery	3×10^{-8}	—	81
	ear artery	3×10^{-7}	—	81

Table 1 *(Continued)*

CAt(M)	Vessels compared	Modes of activation		References
Diltiazem	Rabbit aorta	$>10^{-4}$	8×10^{-7}	24
	mesenteric artery	2×10^{-7}	$3\text{–}6 \times 10^{-7}$	39, 82
	mesenteric resistance vessels	1×10^{-8}	6×10^{-7}	87
	basilar artery	1×10^{-8}	1×10^{-7}	82
	ear artery	1×10^{-6}	2×10^{-6}	82
	renal artery	1×10^{-4}	4×10^{-5}	82
		$PGF_{2\alpha}$		
	Dog cerebral artery	5×10^{-6}	—	86
	mesenteric artery	2×10^{-5}	-	86
		NE		
	Rat mesenteric bed	10^{-4}	6×10^{-6}	83
Nisoldipine	Rabbit aorta	$>10^{-4}$	1×10^{-9}	26, 78
	mesenteric artery	2×10^{-8}	3×10^{-9}	36
Flunarizine	Rabbit ear artery	—	3×10^{-8}	92
(ug/ml)	Dog basilar artery	—	0.05	90
	carotid artery	—	0.2	90
	saphenous vein	—	0.3	90
	splenic artery	—	0.5	90
	coronary artery	—	2.0	90
	tibial artery	—	>10	90
	Rat aorta	2×10^{-7}	2×10^{-8}	84
	mesenteric artery	2×10^{-8}	2×10^{-9}	92
	caudal artery	—	1×10^{-7}	92
		5HT		
Nimodipine	Rabbit basilar artery	7×10^{-10}	2×10^{-10}	88
	saphenous artery	$>10^{-5}$	3×10^{-10}	88
		TXA_2		
	Rabbit basilar artery	1×10^{-9}	—	94
	saphenous artery	$>>10^{-8}$	—	94
		$PGF_{2\alpha}$		
	Human pial artery	2×10^{-8}	2×10^{-9}	85
	omental artery	—	7×10^{-9}	85

[a] IC_{50} = concentration of Ca antagonist (CAt) required to produce 50% relaxation or inhibition of contraction.

VARIABILITY OF SENSITIVITY OF POCs TO CA ANTAGONISTS AMONG VASCULAR BEDS When vascular tissues are contracted with a depolarizing K solution, there appears to be a similarity among vascular beds in their sensitivities to inhibition by CAts (see Table 1). K-induced

contractions of rat aorta and superior mesenteric artery are equisensitive to nifedipine (84), and are similar in sensitivity to cinnarizine (80); canine coronary, cerebral, and mesenteric arteries are about equisensitive to verapamil (86); rabbit aorta and superior mesenteric artery are about equisensitive to D600, nifedipine (79), diltiazem, and nisoldipine (26, 36, 39); rabbit basilar and saphenous arteries (88) as well as human pial and omental arteries (85) are equisensitive to nimodipine; human mesenteric arteries and veins are equisensitive to nifedipine and verapamil (52); and rabbit aorta and canine coronary are equisensitive to D600 (89). Since K-induced contractions are dependent on Ca entry, these studies all suggest that the POC maintains a rather constant sensitivity to CAts throughout the vasculature.

There are two obvious exceptions to this generalization. Van Neuten & Vanhoutte (90) have shown that the IC_{50}s (concentrations required to produce 50% inhibition of activation) for flunarizine inhibition of Ca-induced contractions of isolated depolarized canine vessels were about 0.05, 0.2, 0.3, 0.5, 2, and greater than 10 ug/ml for the basilar artery, internal carotid artery, saphenous vein, splenic artery, coronary artery, and tibial artery, respectively. Cinnarizine has been similarly shown to vary markedly throughout the vasculature in its potency in inhibiting K-induced contractions (80, 91). This variability in POC sensitivity may be peculiar to the diphenylpiperazine CAts. These compounds appear to be somewhat unusual among the CAts. For example, they do not inhibit spontaneous activity of the rat portal mesenteric vein, whereas other CAts do (92). A second exception is seen in the case of the cerebral circulation. Nifedipine has been shown to be somewhat more potent in inhibiting K-induced contractions in human pial arteries than in omental arteries (85). Rabbit basilar artery is more sensitive to D600 (81) than mesenteric artery or aorta (79).

VARIABILITY OF ROC CA ANTAGONIST SENSITIVITY AMONG VASCULAR BEDS The sensitivity of Ne-induced contractions to inhibition by CAts varies more markedly among vascular tissues than does that of K-induced contractions (see Table 1). For example, the IC_{50} for cinnarizine inhibition of Ne-induced contraction of rabbit mesenteric artery is about 10^{-5} M. This concentration has no effect on contraction of rabbit aorta (80). Diltiazem, nifedipine, D600, and nisoldipine are also more potent in rabbit mesenteric artery than in aorta (24, 36, 39, 78, 79). Ne-induced contractions of canine coronary arteries are much more sensitive to inhibition by D600 than those of rabbit aorta (89). Flunarizine is about ten times more potent, and cinnarizine about twice as potent in inhibiting Ne-induced contractions in rat superior mesenteric artery than in aorta (93). Diltiazem is approximately 10,000-fold more potent in inhibiting Ne-induced contraction in rabbit mesenteric resistance vessels than in aorta (24, 87).

Sensitivity of ROCs to CAts is more directly indicated by measuring inhibition of ^{45}Ca uptake or unidirectional influx. Godfraind & Dieu (93) have shown that Ne-dependent ^{45}Ca uptake as well as contraction is about 10 times more sensitive to inhibition by flunarizine in the rat superior mesenteric artery than in the aorta. Cauvin et al (87) have shown that the rabbit mesenteric resistance vessels show nearly complete inhibition of Ne-stimulated ^{45}Ca influx at 10^{-6} M diltiazem. This concentration only inhibits about 10% and 65% of Ne-stimulated ^{45}Ca influx in rabbit aorta (24) and superior mesenteric artery (Cauvin, unpublished results), respectively. The marked sensitivity of the resistance vessel ROCs to CAts cannot be due simply to greater depolarization and an increased recruitment of POCs, because the K-induced contractions of these vessels are approximately 60 times less sensitive to diltiazem than the Ne-induced contractions (87).

The sensitivity of ROCs in the cerebral vascular bed has also been studied (see Table 1). Nimodipine has been shown to inhibit effectively thromboxane A_2- and serotonin-induced contractions of rabbit basilar arteries at concentrations that had little effect on the responses of saphenous arteries (88, 94). Shimizu et al (86) have shown that strips of canine cerebral arteries contracted with $PGF_{2\alpha}$ are more sensitive to verapamil, diltiazem, and nifedipine than those of mesenteric arteries. Ne-induced contraction of rabbit basilar artery is more potently inhibited by diltiazem and D600 than that of aorta and mesenteric artery (79, 81, 82). Thus, the ROCs of the cerebral circulation, like the POCs, appear to be more susceptible to the action of CAts.

Effect of Agonist Concentration on the Sensitivity of the ROC to Ca Antagonists

We have seen that ROC sensitivity to CAts varies widely among different vascular smooth muscles. In addition, the ROC sensitivity to CAts is inversely related to the agonist concentration in at least one blood vessel. In the rabbit aorta, the IC_{50}s for diltiazem and nisoldipine inhibition of Ne-induced contraction and ^{45}Ca influx increase dramatically as the [Ne] is increased (56). It could be argued that as the [Ne] increases, more intracellular Ca is released. The resulting phasic contractions would thereby become more resistant to these two CAts, since they have little effect on intracellular Ca release. However, the ^{45}Ca influx that mediates the tonic phase of contraction also becomes more resistant to the CAts with increasing [Ne]. Hence, it appears that the ROCs exhibit multiple activated states characterized by varying susceptibility to CAt blockade. This phenomenon is not observed with varying the degrees of activation of the POC.

DO CA ANTAGONISTS DISTINGUISH BETWEEN α_1- AND α_2-ADRENOCEPTOR ACTIVATION? The discovery of the phenomenon described above may help to resolve a controversy that has arisen recently concerning the specific
and Ca release. Van Meel et al (95–97), Godfraind & Miller (98), and Nghiem et al (99) have recently shown that activation by α_2-adrenoceptor agonists is more readily inhibited by CAts than is activation by α_1-adrenoceptor agonists. These data all support the hypothesis that α_1-adrenoceptors are linked to intracellular Ca release and α_2-adrenoceptors to Ca influx. De Mey & Vanhoutte (100) have reached the opposite conclusion after finding
amine more than those to Ne in both splenic artery and saphenous vein. In order to test the hypothesis that Ca influx
linked to different α-adrenoceptor subtypes, Cauvin et al (107) have studied the inhibition by selective α-adrenoceptor antagonists of Ne-induced contraction, ^{45}Ca influx, and intracellular Ca release in rabbit aorta. Prazosin, an α_1-antagonist, was approximately 1000-fold more potent in inhibiting intracellular Ca release and ^{45}Ca influx
nist. These data led to the conclusion that α_1-adrenoceptor activation of rabbit aorta was responsible for both influx
lar Ca.

Although the three different conclusions reached in the above studies could be the result of species variation, a more plausible explanation may be based on the phenomenon of decreasing CAt sensitivity with increasing [Ne]. This phenomenon necessitates that equieffective agonist concentrations must be used in order to make meaningful comparisons of the effects of CAts on contractions induced by selective α-adrenoceptor agonists. Such concentrations were not used in the studies of De Mey & Vanhoutte (100), Nghiem et al (99), and Godfraind & Miller (98), wherein the contractions that were most sensitive to the CAts were in each case those induced by the least efficacious agonists. Van Meel et al (95–97), on the other hand, did compare the effects of CAts on the in vivo vasoconstriction produced by *equieffective* doses of B-HT920 (α_2-selective) and methoxamine (α_1-selective), in the pithed rat. A significant
in this model, however, appears to be due to stimulation of the renin-angiotensin system, as captopril significantly impairs it. Hence, no conclusions regarding the susceptibility to CAts of vascular postjunctional α_2-adrenoceptor activation can be drawn from these studies. It is concluded, therefore, that neither α_1- nor α_2-adrenoceptor subtypes are specifically linked to one activation process or the other (i.e. Ca influx
Ca release). Differing sensitivities to CAts among selective α-adrenoceptor agonist-induced contractions may merely reflect

between CAt sensitivity of the ROC and degree of activation induced by the α-adrenoceptor agonists.

Theoretical Bases for Ca Antagonist Selectivity

There are three general observations that follow from the above discussion of CAt selectivity: 1. CAts often show greater selectivity for high K-induced over agonist-induced contractions; 2. CAt potency for inhibiting agonist-induced contractions varies widely among vascular tissues; and 3. susceptibility of agonist-induced contractions to CAt inhibition diminishes with increasing levels of agonist activation. We discuss below several factors that may influence CAt selectivity and provide a theoretical basis for these general observations.

CHEMICAL DIFFERENCES IN CA INFLUX PATHWAYS The preliminary results of [^{3}H]-nitrendipine binding studies described above (62–64) are indicative of the CAts acting at specific proteinic membrane structures or channels. The stereospecificity exhibited by CAts (65) also suggests that these compounds interact with specific regions of membrane components capable of maintaining precise tertiary structure. Thus, there is ample evidence to support the concept that at least some of the specificity exhibited by CAts for one type of activation over another or for a particular vascular bed may be due to variations in the chemical structure of Ca influx channels. Indeed, the selective inhibition of K-induced Ca influx over Ne-induced ^{45}Ca influx strongly supports the existence of chemically distinct POCs and ROCs in vascular smooth muscle (76). Similarly, the observation that agonist-induced contractions of the basilar artery are more sensitive to CAts than those of other vessels indicates that the chemical nature of ROCs may differ in different blood vessels (88).

DIFFERING DEGREES OF AGONIST-INDUCED DEPOLARIZATION The possibility that agonist-induced activation of different vessels might involve differing degrees of membrane depolarization might be yet another factor influencing the specificity of CAts for various vascular beds. Since POCs generally exhibit a greater sensitivity for CAts than ROCs, a greater degree of recruitment of POCs would be expected to cause a greater sensitivity to CAts. The extent to which this phenomenon may contribute to the variability of CAt sensitivity of various beds cannot be ascertained at present, owing to the paucity of studies relating CAt sensitivity to the degree of membrane depolarization in smooth muscle.

AGONIST-INDUCED RELEASE OF INTRACELLULAR CA The initial fast (F) phase of the contractile response of conduit arteries to agonists

involves the release of intracellularly bound Ca and is thus insensitive to Ca entry blockade (10, 18, 32, 33). In the mesenteric vascular bed, with increasing branching of the arterial tree, the degree of agonist-induced release of intracellular Ca decreases (87). At the level of the resistance vessel (circa 200 micron ID), agonist-induced activation is mediated solely by a stimulation of Ca influx and no intracellular Ca release occurs. Similarly, activation of both conduit arteries and resistance vessels by high K is mediated entirely by a stimulation of Ca entry and does not involve the release of intracellularly bound Ca stores (24, 87). Thus, the release of intracellular Ca may explain why the initial phasic component of the contractile response of conduit arteries is insensitive to CAts. However, this explanation does not apply to the sustained phases of agonist-induced contractions, which are completely dependent on Ca entry.

SEQUESTRATION BY THE AGONIST RELEASABLE CA STORE (ARC)

While the tonic phases of the Ne- and K-induced contractions are both mediated by an entry of extracellular Ca, these two types of activation may differ in the extent to which intracellular Ca sequestration occurs. Ca uptake into the ARC has been demonstrated to attenuate the contractile response to the Ca influx stimulated by high K (26). During agonist-induced activation, however, not only is Ca released from the ARC, but the ability of this pool to act as an intracellular Ca sink is also reduced (26). The Ca influx that occurs during agonist-induced activation may, therefore, be utilized more effectively than that occuring during high K activation. Accordingly, the magnitude of the relaxation resulting from a partial blockade of the K-stimulated Ca influx may be more pronounced than that resulting from a similar blockade of the Ne-stimulated Ca influx. As mentioned above (see section on inhibition of intracellular Ca release), the ARC may also play a role in sequestering Ca that enters the cell through the passive leak pathway. An agonist-induced reduction in the sequestering capacity of the ARC may allow the Ca that enters via this leak pathway to contribute to tension development. This concept would explain the residual, sustained contracture that persists after the agonist-stimulated Ca influx has been completely blocked by CAts (26, 93). Thus, in vessels possessing an ARC, there may be a component of the tonic phase of the agonist-induced contraction that is supported by the passive Ca leak. The insensitivity of the leak pathway to CAts (23, 26) could contribute to reduced sensitivity to CAts exhibited by these vessels when contractions are elicited by agonists capable of releasing the ARC. Similarly, since low concentrations of Ne do not release Ca from the ARC, the above phenomenon may also contribute to the inverse relationship between Ne concentration and CAt sensitivity of Ne-induced tension.

MULTIPLE ACTIVATED STATES OF THE ROC The diversion of incoming Ca into the ARC during activation with low but not high Ne concentrations does not explain why ^{45}Ca *influx* induced by lower Ne concentrations is more sensitive to CAt inhibition than that induced by higher concentrations (24, 56). This observation suggests multiple activated states of the ROC which are characterized by different sensitivities to CAts. These activated states do appear to be somehow related to the degree to which Ne releases Ca from the ARC, since the CAt sensitivity of the ROC decreases as Ne-induced intracellular Ca release increases (56). An analogous observation has been made (87) in that the sensitivity of Ne-induced ^{45}Ca influx to diltiazem increases from rabbit aorta to superior mesenteric artery, to mesenteric resistance vessels, while the release of intracellular Ca by Ne decreases from the proximal to distal arteries. CAt sensitivity of K-induced ^{45}Ca influx, on the other hand, remains the same for varying K concentrations in rabbit aorta (56), and from proximal to distal arteries (87).

In an attempt to explain these phenomena, the following working model is proposed (see Figure 1). The POCs behave in a simple way, being closed at rest and open during depolarization, whereas the ROCs exhibit at least two different open states in addition to their resting closed state. The open state of the POC is sensitive to CAt blockade, as is one of the Ne-activated open states of the ROC. This CAt-sensitive open state of the ROC is seen at *low* [Ne] in arteries capable of intracellular Ca release from the ARC. It is also seen at all [Ne] in arteries in which Ne does not induce intracellular Ca release. A second open state, which is insensitive to CAt blockade, is seen at higher [Ne] in larger arteries when Ne induces intracellular Ca release from the ARC. If the ARC consists of plasma membrane-bound Ca (18, 102, 103), its release may destabilize the membrane, causing insensitivity of the ROC to CAts. On the other hand, the release of Ca from the ARC may not cause the CAt insensitivity of the ROC; it may simply occur concomitantly. Putney et al (104) have presented evidence that acetylcholine-induced conversion of phosphatidylinositol to phosphatidic acid (PA) is associated with intracellular Ca release and increased Ca influx in the parotid gland. The PA is postulated to function as a Ca ionophore (104), which has been shown to be insensitive to CAts (28). The interesting possibility thus exists that at high [Ne] in large arteries, both intracellular Ca release and CAt-insensitive stimulated Ca influx are a consequence of conversion of phosphatidylinositol to phosphatidic acid (PA).

It is plausible, therefore, that the susceptibility of agonist-induced tonic contractions in a given vascular smooth muscle to inhibition by CAts may be predicted to be inversely related to the degree to which the agonist releases intracellular Ca in that tissue. From studies that provide information on agonist-induced release of Ca from the ARC, this prediction appears

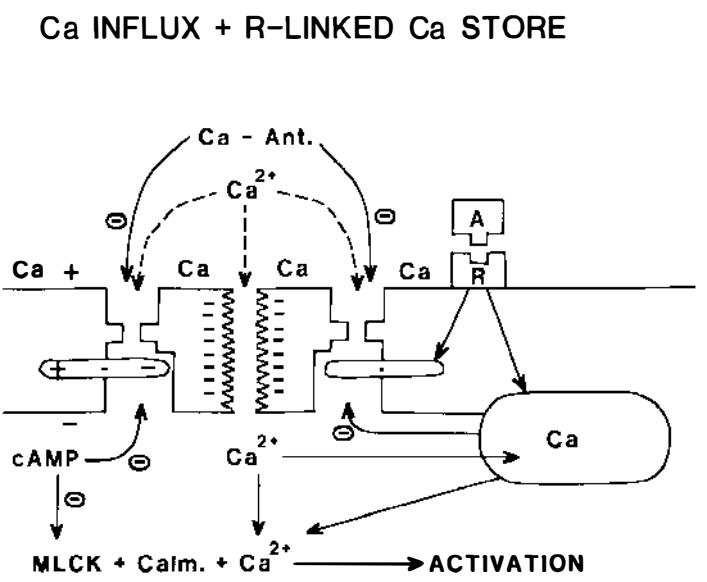

Figure 1 Schematic diagram of pathways for Ca^{2+} delivery to the myoplasm. Ca influx is mediated (from left to right) by potential operated Ca channels (POCs), a Ca leak pathway, and receptor operated Ca channels (ROCs). Ca^{2+} antagonists block the POCs and ROCs, but not the leak. Receptor (R) occupation by agonists (A) also induces the release of an intracellular agonist-releasable Ca fraction (ARC). Release of the ARC appears to be related to a reduction of ROC sensitivity to CAts. When not released the ARC also functions in Ca removal from superficial cytoplasm. cAMP appears to have multiple relaxing effects on POCs, sarcoplasmic reticulum, and myosin light chain kinase (MLCK).

to be borne out (36, 39, 78, 80–82, 89, 94). However, there are some studies wherein contractions that involve agonist-induced release of intracellular Ca are *more* susceptible to CAts than those that do not (52, 84). In particular, the venous system of the vasculature may be an area where the proposed framework for predicting CAt sensitivity does not apply. Ca influx-dependent contractions induced by agonists in the facial (105) and portal vein (106) are quite resistant to CAts. These CAt-resistant modes of activation in veins emphasize the necessity to clarify the mechanisms of activation of each particular vascular smooth muscle before a genuine understanding of the mechanism(s) of action of inhibitory agents such as the CAts can be achieved.

CONCLUSIONS

We focused in this review on the interactions of CAts with vascular smooth muscle cells. It became clear that knowledge of Ca transport processes

located in the plasmalemma and in intracellular organelles is essential to understanding these interactions. Overwhelming evidence supports the theory that CAts inhibit plasmalemmal Ca channels that are activated by depolarization and receptor occupation. Nevertheless, intracellular Ca movements do affect the nature of the vasodilatory response to CAts.

We hope that the evidence and theoretical considerations discussed above will be helpful in the design of new experiments and in providing some rationale (though at present still incomplete) for therapeutic applications. CAts are presently used to treat many vascular disorders, which include Prinzmetal's variant and typical angina, cerebral vasospasms, Reynaud's disease, migraine headaches, and hypertension. New theoretical guidelines are needed in order to develop agents with higher degrees of selectivity, and to predict CAt interactions with other vasoactive agents.

ACKNOWLEDGMENTS

We are grateful to Barbara Bradie for typing and editing this review, and for financial support to the NIH grants HL 2467 and HL 07188 and the Florida Heart Association grant 7/82 F166(2).

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