Role of L-type calcium channels and PKC in active tone development in rabbit coronary artery

Caroline A. Cobine,* Brid P. Callaghan,* and Kathleen D. Keef

Department of Physiology and Cell Biology, University of Nevada, Reno, Nevada

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Cobine CA, Callaghan BP, Keef KD. Role of L-type calcium channels and PKC in active tone development in rabbit coronary artery. Am J Physiol Heart Circ Physiol 292:H3079–H3088, 2007. First published February 2, 2007; doi:10.1152/ajpheart.01261.2006.—The present study investigated active tone development in isolated ring segments of rabbit epicardial coronary artery. Endothelium-denuded arteries (E−) or endothelium-intact (E+) vessels treated with the NO synthase inhibitor Nω-nitro-1-arginine (100 μM) developed active tone, which was enhanced by stretch and reversed by the NO donor sodium nitroprusside (SNP; IC50 = 9 nM). Nifedipine abolished active tone and the contractile response to phorbol dibutyrate (PDBu; 10 nM) with the same potency (IC50 = 8 nM), whereas 300 nM PDBu responses were only partially blocked by nifedipine. The classical and novel PKC inhibitors GF-109203X (IC50 = 1–2 μM) and chelerythrine (IC50 = 4–5 μM) and the classical PKC inhibitor Gö-6976 (IC50 = 0.3–0.4 μM) blocked both active tone and 10 nM PDBu responses with similar potency. Active tone development was associated with depolarization of endothelial cell membrane potential (Em) and a shift to the left of the Em-vs.-contraction relationship determined by varying extracellular potassium. The depolarization and leftward shift were reversed by either chelerythrine (10 μM) or SNP (30 nM). PDBu (100–300 nM) increased peak L-type calcium channel (CaL) currents in isolated coronary myocytes, and this effect was reversed by chelerythrine (1 μM) or Gö-6976 (200 nM). SNP (500 nM) reduced CaL currents only in the presence of the PKA blocker 8-bromo-2′-O-monoethyl-cAMPs, Rp isomer (10 μM). In conclusion, active tone development in coronary artery is suppressed by basal NO release and is dependent on both enhanced CaL activity and classical PKC activity. Both Em-dependent and -independent processes contribute to contraction. Our results suggest that one Em-independent process is direct enhancement of CaL activity by PKC.

vascular smooth muscle; endothelium; nitric oxide; protein kinase C; calcium channels; patch clamp; membrane potential

THE MAJOR EPICARDIAL CORONARY arteries of the heart, unlike resistance vessels, are generally reported to remain quiescent following stretch when the endothelium is intact (17). However, recently we have found that either mechanical removal of the endothelium or addition of the NO synthase (NOS) inhibitor Nω-nitro-1-arginine (L-NNA) to endothelium-intact coronary arteries leads to active tone development. A similar phenomenon has been reported for the main coronary arteries of the rat (19). Little is known about the mechanisms underlying this active tone in larger coronary vessels.

Myogenic tone in microvessels has been studied extensively, and it has been linked to both increased L-type calcium channel (CaL) activity (34, 38, 45) and PKC activity (15, 21, 22, 26, 37, 39, 52, 64). In the present study, we undertook experiments to examine the role of CaL and PKC in active tone development in the coronary artery. CaL is a voltage-dependent conductance that opens in response to membrane depolarization, leading to calcium entry and contraction. In contrast, PKC can cause contraction in the absence of a change in membrane potential (Em) by increasing the sensitivity of the myofilaments to calcium (58). However, the actions of PKC can also be linked to CaL activity. For example, PKC can modify the activity of other ion channels that depolarize the membrane and therefore increase CaL activity (16, 52). Furthermore, PKC can have direct excitatory effects upon CaL (6, 8, 55). Thus the manner in which CaL and PKC contribute to the regulation of active tone may be interdependent.

In this study, we have examined the hypothesis that active tone in the coronary artery is dependent on both PKC and CaL activity and that PKC is linked to CaL via changes in Em as well as direct effects of PKC on CaL. To explore this hypothesis, contraction and Em were measured in intact segments of coronary artery and CaL currents were measured in isolated myocytes by using the patch-clamp technique. Because little is known about active tone development in larger coronary arteries, initial studies characterized the dependence of active tone on endothelial-derived NO and stretch. In subsequent experiments, we addressed the role of CaL and PKC by using a variety of pharmacological blockers and activators of PKC and CaL. Because the direct effects of PKC on CaL are predicted to increase the amount of calcium entry and contraction occurring at given level of Em, we also examined the relationship between Em and contraction in the presence and absence of active tone. Finally, the direct effects of PKC on CaL currents were evaluated with the PKC activator phorbol dibutyrate (PDBu). Our results suggest that both PKC and CaL contribute to active tone development in the rabbit coronary artery and that PKC may be linked to CaL activity via both direct effects on the channel and indirect effects mediated via other channels that depolarize the membrane.

METHODS

Contractile experiments. Male albino rabbits (2.3–2.9 kg; Western Oregon Rabbitry, Philomath, OR) were killed with an overdose of 1 ml Euthasol solution (0.39 g pentobarbital sodium and 0.05 mg phenytoin sodium; Virbac AH, Fort Worth, Texas) injected into the ear vein via a protocol approved by the University Animal Care and Use committee. The left descending coronary artery and its main branches were dissected free and cleared of cardiac muscle and fat in ice-cold Krebs-Ringer bicarbonate solution (KRBS) of the following composition (in mM): 118.5 NaCl, 4.7 KCl, 1.2 MgCl2, 23.8...
ATP-Na2 and were incubated between 20 and 25 min with gentle agitation at 37°C, then rinsed four times with Hanks’ solution (0.1 mM Ca2+). After completion of the digestion, single cells were dispersed by gentle trituration of the segments with a wide-tipped fire-polished Pasteur pipette. The cell suspension was stored at 2°C in Hanks’ solution (0.1 mM Ca2+) containing 2 mg/ml trypsin inhibitor (Sigma) and 2 mg/ml BSA (Sigma) and was used within 6 h.

For experiments examining stretch, E+ vessels were equilibrated for 2.5 h as described above. After equilibration, passive tension was reduced from 0.5 g to a minimum level (i.e., ~0.05 g). This was followed by one of two protocols. In protocol 1, after 5 min l-NA (100 µM) was added. After development of a stable level of active tone, vessels were stretched by applying 0.5 g of tension. In protocol 2, after 30 min vessels were stretched by applying 0.5 g of tension. In preliminary experiments on E+ vessels, we found that 0.5 g of applied tension was sufficient to produce a maximum response to 90 mM KCl, whereas when tension was reduced to 0.05 g, the response to 90 mM KCl was reduced to 45% of maximum.

Intracellular measurements of $E_m$. For microelectrode measurements, 3-mm-long ring segments of coronary artery were mounted in a 2-ml bath superfused with KRBS and maintained at 37°C. The vessel was attached to a tension transducer via a triangle inserted through the lumen. A second wire was inserted through the vessel lumen and stably mounted to the base of the chamber. A resting tension of 0.5 g was applied to vessels. Vessels were equilibrated for 2 h followed by addition of combined AEP (30 µM) and KCl (90 mM) to generate a maximum contraction. Experiments were begun 30 min after washing out this solution. $E_m$ was measured using sharp microelectrodes with resistances ranging from 60 to 100 MΩ. Impalements were judged based on a rapid negative deflection from 0 mV following a tap, a stable level of potential while in the cell, and a rapid return to near the original 0-mV potential on removal of the electrode from the cell. It was generally possible to maintain impalements during the onset of contraction or relaxation; thus several impalements were made during the control period and following establishment of a new condition. $E_m$ values for a given condition were averaged. An example of this approach is shown in RESULTS (see Fig. 6). $E_m$ was modified by either raising extracellular potassium concentration ([K+]o) from 6 mM to 16 or 26 mM or by addition of pinacidil (0.3 µM).

Isolation of coronary artery myocytes. Left and right descending coronary arteries were removed and cleared of cardiac muscle and fat in ice-cold KRBS aerated with 95% O2 and 5% CO2. Vessels were cut into small segments and placed in Ca2+-free Hanks’ solution (in mM: 125 NaCl, 5.4 KCl, 15.5 NaHCO3, 0.34 Na2HPO4, 0.44 KH2PO4, 10 glucose, and 2.9 sucrose, aerated with 95% O2 and 5% CO2) for 30 min at 37°C. The segments were then transferred to Hanks’ solution (0.1 mM Ca2+) containing 1 mg/ml collagenase type I (Sigma, St. Louis, MO), 0.5 mg/ml protease type XXVII (Sigma), 2 mg/ml trypsin inhibitor (Sigma), 2 mg/ml BSA (Sigma), and 0.1 mg/ml ATP- Na2 and were incubated between 20 and 25 min with gentle agitation at 37°C, then rinsed four times with Hanks’ solution (0.1 mM Ca2+). After completion of the digestion, single cells were dispersed by gentle trituration of the segments with a wide-tipped fire-polished Pasteur pipette. The cell suspension was stored at 2°C in Hanks’ solution (0.1 mM Ca2+) containing 2 mg/ml trypsin inhibitor (Sigma) and 2 mg/ml BSA (Sigma) and was used within 6 h.
RESULTS

Active tone develops following blockade of endothelial NOS activity and is reversed when NO is reintroduced. E+ rabbit coronary artery segments did not develop active tone throughout the duration of a 6-h experiment even when periodically stimulated with the histamine H₁ receptor agonist AEP (Fig. 1Aa). However, in E− vessels, tone developed following 1.5–3 h of equilibration and persisted in spite of repeated washes of the vessel with fresh KRBS (Fig. 1Ab, n = 17). Active tone also developed in E− vessels in which no contractile stimulus was added during the initial equilibration time (Fig. 1B). Since NO is a well-established factor released from the endothelium, we examined whether tone could be generated in E+ vessels by inhibition of NOS activity. Addition of the selective endothelial/neuronal (e/nNOS) inhibitor L-NNA (100 μM) to E+ vessels resulted in active tone development (Fig. 1C), which averaged 30.1 ± 3.2% of a maximum contraction (n = 45), whereas the selective inducible NOS inhibitor L-NIL (100 μM) was without effect (n = 4) (5). Tone also developed with a second selective e/nNOS inhibitor, L-NAME (300 μM, n = 4) (5). The amplitude of active tone with L-NNA addition to E+ vessels was not significantly different from the active tone that...
developed in E− vessels (37.1 ± 3.6% maximum, n = 17). Active tone also occurred with NOS blockade or in E− vessels when bathed with indomethacin (10 μM, n = 16), suggesting that tone was not due to generation of a cyclooxygenase product.

Because blocking NOS activity with L-NNA led to active tone development, we investigated whether tone could be reversed by addition of the NO donor SNP. Interestingly, active tone was reversed by nanomolar concentrations of SNP (IC50 = 9 nM) (Fig. 1D).

Active tone enhances potassium-induced contractions and is abolished by pinacidil. The active tone that develops with addition of L-NNA was further investigated by depolarizing Em with high [K]o or by hyperpolarizing E_m with the ATP-sensitive potassium channel (KATP) opener pinacidil. The entire [K]o-vs.-contraction relationship, including the peak response, was shifted upward in vessels with L-NNA-induced tone, suggesting that an E_m-independent process(s) contributes to active tone development (Fig. 2A). In contrast, tone was entirely reversed with pinacidil (Fig. 2B; IC50 = 33 nM), suggesting that an E_m-dependent process(s) also contributes to active tone development. No further relaxation occurred when the KRBS was switched to a Ca2+ free 1 mM EGTA solution, suggesting that the remaining baseline tension was passive (0.48 ± 0.03 vs. 0.46 ± 0.03 g baseline tension before and after Ca2+ free solution, respectively; n = 6).

Stretch elicits an active response following NOS blockade. To examine whether stretch contributes to active tone development, we carried out experiments in which NOS activity was blocked before applying stretch to the tissue (see METHODS). Addition of L-NNA to minimally stretched vessels (~0.05 g applied tension) gave rise to a small increment of active tone (Fig. 3A). However, when tension was subsequently raised 30–45 min later by 0.5 g, a significantly greater amount of active tone developed (Fig. 3A). In contrast, when vessels were stretched by 0.5 g in the absence of L-NNA, tone did not develop, although subsequent addition of L-NNA generated active tone in these vessels (Fig. 3B). The effects of stretch on L-NNA-induced tone are summarized in Fig. 3C.

PDBu-induced contractions and active tone are both reversed with nifedipine and PKC inhibitors. To investigate the role of PKC in active tone development, we first characterized the effects of the PKC activator PDBu. PDBu produced concentration-dependent contraction of tissues with an EC50 of 7 nM (Fig. 4, A and B). The rate at which contraction developed with low concentrations of PDBu was considerably slower than with higher concentrations of PDBu (Fig. 4, A and C).

To determine the extent to which contractile responses elicited by stimulating PKC activity with PDBu resemble active tone development, we compared the pharmacology of these two types of contraction. The dihydropyridine CaV blocker nifedipine (1 μM) completely blocked L-NNA-induced active tone as well as the response to a low concentration of PDBu (10 nM) (Fig. 4D) with equal potency (IC50 = 9 nM) (Fig. 4E). In contrast, the contraction elicited with a higher concentration of PDBu (300 nM) was only partially reversed with nifedipine (Fig. 4E). Thus CaV activity plays an important role in active tone and the response to 10 nM PDBu, whereas with higher PDBu concentrations other mechanisms, such as calcium sensitization (7, 33, 50), play an increasingly greater role. Both chelerythrine and GF-109203X produced concentration-dependent inhibition of active tone and responses to PDBu (10 nM). There was no significant difference in the effects of either drug on active tone vs. PDBu
responses (GF-109203X IC$_{50}$ = 2.4 and 1.1 µM, respectively; chelerythrine IC$_{50}$ = 4.2 and 4.6 µM, respectively) (Fig. 5, A and B). Interestingly, both types of contraction were also blocked with equal potency by the classical PKC inhibitor Gö-6976 (IC$_{50}$ = 0.4 and 0.3 µM, respectively) (Fig. 5C).

The relationship between $E_m$ and contraction is shifted to the left during active tone development and shifted back with chelerythrine or SNP. To evaluate the extent to which $E_m$-dependent and -independent processes contribute to active tone generation, we measured $E_m$ as a function of contraction under various conditions. An example of several recordings made during active tone development is shown in Fig. 6A. Resting $E_m$ in control vessels averaged −56 ± 0.8 mV ($n$ = 15). In the presence of l-NNA, $E_m$ was significantly depolarized compared with control and averaged −48 ± 0.7 mV ($n$ = 29). Addition of either SNP (30 nM) or chelerythrine (10 µM) in the presence of l-NNA repolarized cells to −52.5 ± 0.8 mV ($n$ = 7) and −53.5 ± 1.6 mV ($n$ = 8), respectively (Fig. 6B).

To evaluate the relationship between $E_m$ and contraction in the presence and absence of tone, $E_m$ was varied by increasing [K]$_o$ from the control level (6 mM) up to 26 mM (Fig. 7A). Figure 7B plots the $E_m$-vs.-contraction relationships associated with the four conditions listed in Fig. 7A. This procedure revealed that the $E_m$-vs.-contraction relationship was shifted to the left when basal NO release was blocked with l-NNA. For example, during control conditions when basal NO release was present, raising [K]$_o$ to 16 mM depolarized cells to −47.7 ± 1.1 mV and caused very little contraction (6.0 ± 2.5% maximum). In contrast, blocking NOS with l-NNA led to a significant depolarization of $E_m$, whereas both SNP (30 nM) and chelerythrine (10 µM) caused significant repolarization in the continued presence of l-NNA (nonpaired t-test, $P < 0.05$). Values are means ± SE.

These data suggest that both NO and PKC contribute to the regulation of active tone and that both $E_m$-dependent and $E_m$-independent effects are involved.

Fig. 5. PKC blockers reverse both active tone and the response to 10 nM PDBu. Concentration-dependent effects of GF-109203X (A), chelerythrine (B), and Gö-6976 (C) on PDBu (■) and active tone (▲) elicited with l-NNA in E$^+$ vessels are shown. There was no significant difference in PKC blocker effects on active tone vs. PDBu responses (ANOVA, $P > 0.05$). Values are means ± SE.

Fig. 6. Effect of l-NNA, SNP, and chelerythrine on membrane potential ($E_m$). A: sample traces showing simultaneous measurement of $E_m$ (top trace) and contraction (bottom trace) as a function of time. Four impalements are shown in this trace, two during the control period and two following addition of l-NNA. Each impalement was associated with a rapid drop in $E_m$, whereas loss of impalement or removal of the electrode from the cell was seen as a rapid return of potential toward 0. Active tone was associated with a small depolarization of $E_m$. The final impalement was lost during the onset of relaxation initiated with SNP. B: changes in $E_m$ associated with various conditions. l-NNA led to a significant depolarization of $E_m$, whereas both SNP (30 nM) and chelerythrine (10 µM) caused significant repolarization in the continued presence of l-NNA (nonpaired t-test, $P < 0.05$). Values are means ± SE.

Fig. 7. Activation of PKC increases $Ca^{2+}$ currents. The above experiments revealed that blockade of PKC with chelerythrine...
inhibited active tone and shifted the \( E_m \text{-vs.-} \) contraction relationship to the left. Thus PKC contributes to active tone and acts in part via \( E_m \)-independent mechanisms. One possible \( E_m \)-independent mechanism is direct upregulation of \( C_a \) activity by PKC. To explore this possibility, we recorded \( C_a \) currents in coronary artery myocytes and examined the effects of the PKC activator PDBu. Currents were elicited by stepping voltage from a holding potential of \(-70\) to \(0\) mV for \(370\) ms at \(30\)-s intervals. PDBu at concentrations of \(100\) nM or greater gave rise to a significant increase in \( C_a \) current. An example of the effect of PDBu on currents is shown in Fig. 8A, and the mean increase in current amplitude is plotted in Fig. 8B. The stimulation of currents with PDBu was reversed with either chelerythrine (1 \( \mu \)M; Fig. 8C) or Go\textsuperscript{-6976} (200 nM; Fig. 8D). PDBu (10 nM) did not produce a detectable increase in \( C_a \) current. This result is not surprising given the extremely long time course required for development of the 10-nM PDBu response in isolated tissues (see Fig. 4).

The NO donor SNP reduces \( C_a \) currents when PKA is inhibited. In contrast to PKC, the NO-cGMP-PKG pathway causes inhibition of \( C_a \) currents in various smooth muscles (1, 4, 9, 54). Because removal or addition of NO also shifted the \( E_m \text{-vs.-} \) contraction relationship, we reasoned that, like PKC, NO may have direct effects upon \( C_a \). However, concentrations of SNP up to \(500\) nM did not modify \( C_a \) currents in isolated coronary artery myocytes. Because cGMP can also cross-activate PKA (49), we completed additional experiments examining the effects of SNP in the presence of the PKA blocker Rp-8-Br-MB-cAMPS (10 \( \mu \)M). Inclusion of Rp-8-Br-MB-cAMPS in the superfusate did not significantly affect \( C_a \) currents, but addition of 500 nM SNP in the presence of Rp-8-Br-MB-cAMPS now led to a 54% reduction of \( C_a \) current amplitude, whereas 100 nM SNP was still without effect (Fig. 9).

**DISCUSSION**

Active tone development in rabbit epicardial coronary arteries occurs when the ongoing release of NO from the endothelium is blocked. It is stretch sensitive and associated with both enhanced PKC and \( C_a \) activity. In addition, several lines of evidence suggest that direct modulation of \( C_a \) activity by PKC may contribute to active tone development in the coronary artery. These points are discussed individually below.

**Role of NO in the regulation of active tone development.** Endothelium-derived NO exerts a dilating influence that opposes arterial constriction in most vascular beds. The actions of NO are typically studied by applying drugs such as acetylcholine or bradykinin, which stimulate the synthesis and release of NO from the endothelium. However, NO is also released in vivo in response to shear stress (48), and some studies (e.g., Ref. 27) report basal release of NO from the endothelium in the absence of either agonist stimulation or shear stress. In this study, we found that active tone developed in the rabbit coronary artery when NOS activity was blocked, indicating...
that spontaneous contraction was opposed by basal NO release. A similar relationship between basal NO release and active tone has been described for the main coronary arteries of the rat (19) and for the guinea pig spiral modiolar artery (27). In the rabbit coronary artery, active tone was also reversed by very low concentrations of the NO donor SNP (IC₅₀ = 9 nM), again suggesting that low background levels of NO can suppress active tone. These data all support the increasingly well-recognized concept that ongoing release of NO from the endothelium significantly contributes to the regulation of vascular reactivity.

In this study, various experiments were undertaken to evaluate the mechanism(s) by which NO modulates tone. The major target of NO is guanylyl cyclase, which in turn generates cGMP, leading to activation of cGMP-dependent protein kinase (PKG) (41). This pathway may cause smooth muscle relaxation via a variety of mechanisms, including 1) changes in ionic conductance that hyperpolarize Eₘ, leading to closure of Cav (19, 23, 44, 56, 63, 66), 2) direct inhibition of Cav, (4, 9, 25, 42, 54), 3) decreased Ca²⁺ sensitivity of the myofilaments (40, 61), 4) increased uptake of Ca²⁺ into the sarcoplastic reticulum (11, 14, 35), and 5) suppression of PKC activity (29, 51, 53). Because NO removal caused depolarization and NO addition caused repolarization, the actions of NO were likely to be due in part to changes in Eₘ. Indeed, our observation that tone was entirely reversed by the KₐTP channel opener pinacindil further supports the notion that active tone can be modulated by changes in Eₘ. However, NO addition and removal also shifted the relationship between Eₘ and contraction, suggesting that additional Eₘ-independent mechanisms were involved as well. The fact that the maximum KCl-induced contraction was smaller when basal NO release was present also suggests that NO suppresses contraction in part via one or more Eₘ-independent mechanisms (i.e., mechanisms 2–5).

To investigate the possible direct effects of NO on Cav (mechanism 2) we undertook patch-clamp studies on isolated rabbit coronary artery myocytes. These studies revealed that even a high concentration of SNP (500 nM) was without effect on Cav currents unless applied in the presence of a PKA inhibitor. Higher concentrations of cGMP can cross-activate PKA (3, 49, 62), which in turn can enhance Cav currents (see Ref. 30). Thus blockade of PKA may reveal SNP-induced inhibition of Cav current because it eliminates the opposing effects of cGMP on PKA. However, since both PKA blockade and 500 nM SNP were required to observe Cav inhibition, our results do not provide a particularly compelling case for direct inhibition of Cav by basally released NO in the rabbit coronary artery. Clearly, additional experiments are required to clarify this point.

Role of stretch in active tone development. The active tone characterized in this study was predominantly observed in stretched vessels. However, stretch alone was not sufficient to generate active tone when basal NO release was present. This suggests that two conditions are necessary for optimal tone development, i.e., 1) the absence of basal NO release and 2) stretch. A number of ionic conductances in vascular smooth muscle that can cause depolarization are stretch sensitive (55, 60). In addition, stretch has been linked to PKC activation (64, 65). Thus stretch may initiate changes in the vessel that predispose it toward tone development, whereas NO counter-balances these effects. This interplay of factors favoring and
opposing contraction is discussed further in subsequent sections.

Interestingly, stretch did not cause active tone development immediately following immersion of E− vessels in warm KRBS (e.g., Fig. 1B). The absence of an initial stretch response was likely due to recovery of the vessel following dissection in cold KRBS. Sodium pump activity is temperature dependent, and dissection in cold KRBS will block the pump, causing rundown of ionic gradients. In previous studies (32), we have shown that rewarining the coronary artery after prolonged cold exposure leads to an initial period of hyperpolarization due to sodium pump stimulation, followed by return of $E_m$ to a less polarized value after ~1.5–2 h. A similar transient hyperpolarization can occur when sodium pump activity is blocked by removing potassium from the bathing solution for 30 min and then returning potassium (31). Since active tone is dependent on $C_{av}$ activity and $C_{av}$ activity is $E_m$ dependent, it is possible that tone is suppressed during the initial recovery period because of pump stimulation.

**Role of PKC and $C_{av}$ in active tone development.** Numerous studies (2, 10, 15, 18, 22, 26, 37, 39, 45, 52, 64) have suggested that PKC activity contributes to myogenic tone in microvessels. PKC can cause smooth muscle contraction via a number of different pathways, including 1) changes in ionic conductance that depolarize $E_m$, leading to an increase in $C_{av}$ activity (12, 19, 52); 2) direct stimulation of $C_{av}$ (6, 8, 57), and 3) increased sensitivity of the myofilaments to Ca$^{2+}$ (15, 18, 39).

In coronary artery, chelerythrine reversed active tone development and caused repolarization. This suggests that PKC activity was elevated during active tone development and that the actions of PKC were due in part to effects on $E_m$ (i.e., mechanism 1). Myogenic tone has also been attributed in part to depolarization, which will enhance $C_{av}$ activity (12, 20, 21, 34, 38, 52). Interestingly, myogenic depolarization of rat cerebral arteries is also blocked by chelerythrine (52). This study suggested that PKC activates transient receptor potential channels that depolarize cells, leading to enhanced $C_{av}$ activity, Ca$^{2+}$ entry, and contraction. It is possible that a similar mechanistic contributes to active tone in the rabbit coronary artery. Alternatively, depolarization may involve some other ionic conductance such as delayed-rectifier potassium channels, which are blocked by PKC (12, 13).

Active tone exhibited a number of properties in common with the contraction that developed with the PKC activator PDBu (10 nM). Both contractions were antagonized to the same extent by three different PKC blockers as well as by the $C_{av}$ blocker nifedipine. Interestingly, we observed that the classical PKC antagonist Gö-6976 was an effective antagonist of both types of contraction, suggesting that it is a classical PKC in particular that participates in active tone development and the response to 10 nM PDBu.

**Regulation of $C_{av}$ currents by PKC.** In addition to causing relaxation and repolarization, the PKC inhibitor chelerythrine also shifted the relationship between $E_m$ and contraction to the right, suggesting that PKC modulates tone in part via mechanisms that do not require a change in $E_m$. One such mechanism investigated in this study was the direct effects of PKC on $C_{av}$. Activation of PKC via PDBu led to a significant increase in $C_{av}$ current, which was reversed by either chelerythrine or the classical PKC inhibitor Gö-6976. This is the first time a PKC activator has been shown to directly stimulate $C_{av}$ currents in a coronary myocyte, although PKC activators have been reported to enhance Ca$^{2+}$ currents in several other smooth muscles (6, 8, 57). Previous studies using PKC inhibitors also suggest that PKC can directly enhance Ca$^{2+}$ currents in coronary artery (37, 43). The ability of Gö-6976 to reverse the effect of PDBu is interesting because it suggests that a classical PKC in particular underlies current stimulation. Thus our contractile studies implicate $C_{av}$ and classical PKCs in active tone development, and our patch clamp studies suggest that classical PKCs enhance $C_{av}$ currents. Therefore, we propose that active tone is regulated in part via the direct effects of classical PKCs on Ca$^{2+}$ currents. In studies of the rabbit basilar artery, stretch-induced contraction was specifically attributed to the classical PKC-α (64). In addition, in cerebral artery myocytes PKC-α has been shown to promote clusters of $C_{av}$ channels to operate in a persistent gating mode (46, 47), providing a possible mechanism by which classical PKCs may enhance $C_{av}$ currents in rabbit coronary artery myocytes.

In conclusion, our results suggest that the regulation of active tone in the coronary artery involves a balance between factors that either favor or oppose contraction (Fig. 10). Both PKC and NO participate in this regulation, and $C_{av}$ activity is an important (although not exclusive) target. When the endothelium is intact, the balance favors the quiescent vessel. In contrast, when NOS activity is blocked, the balance shifts toward active tone development. Finally, the quiescent state can be restored if either NO is replaced or PKC activity is blocked. In the healthy coronary artery, the balance of factors favors quiescence. However, in various cardiovascular disease states there is reduced bioavailability of NO (24). Under these conditions, the balance is predicted to shift toward active tone development. Indeed, vasospasm is more prevalent in diseased coronary arteries, and this type of activity can give rise to ischemia, myocardial infarction, and sudden death (36). Thus understanding the mechanisms that underlie active tone development in the coronary artery and the role that NO plays in this process is important to understanding both the normal function of this vessel and the changes that may occur with cardiovascular disease.

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**REFERENCES**


