Isoforms of Cyclic Nucleotide Phosphodiesterase PDE3 and Their Contribution to cAMP Hydrolytic Activity in Subcellular Fractions of Human Myocardium*

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Three isoforms of PDE3 (cGMP-inhibited) cyclic nucleotide phosphodiesterase regulate cAMP content in different intracellular compartments of cardiac myocytes in response to different signals. We characterized the catalytic activity and inhibitor sensitivity of these isoforms by using recombinant proteins. We determined their contribution to cAMP hydrolysis in cytosolic and microsomal fractions of human myocardium at 0.1 and 1.0 μM cAMP in the absence and presence of Ca2+/calmodulin. We examined the effects of cGMP on cAMP hydrolysis in these fractions. PDE3A-136, PDE3A-118, and PDE3A-94 have similar $K_{m}$ and $k_{cat}$ values for cAMP and are equal in their sensitivities to inhibition by cGMP and cilostazol. In microsomes, PDE3A-136, PDE3A-118, and PDE3A-94 comprise the majority of cAMP hydrolytic activity under all conditions. In cytosolic fractions, PDE3A-118 and PDE3A-94 comprise >50% of the cAMP hydrolytic activity at 0.1 μM cAMP, in the absence of Ca2+/calmodulin. At 1.0 μM cAMP, in the presence of Ca2+/calmodulin, activation of Ca2+/calmodulin-activated (PDE1) and other non-PDE3 phosphodiesterases reduces their contribution to <20% of cAMP hydrolytic activity. cGMP inhibits cAMP hydrolysis in microsomal fractions by inhibiting PDE3 and in cytosolic fractions by inhibiting both PDE3 and PDE1. These findings indicate that the contribution of PDE3 isoforms to the regulation of cAMP hydrolysis in intracellular compartments of human myocardium and the effects of PDE3 inhibition on cAMP hydrolysis in these compartments are highly dependent on intracellular [Ca2+] and [cAMP], which are lower in failing hearts than in normal hearts. cGMP may amplify cAMP-mediated signaling in intracellular compartments of human myocardium by PDE3-dependent and PDE3-independent mechanisms.

PDE3 cyclic nucleotide phosphodiesterases are important in the regulation of intracellular cAMP content in cardiac myocytes (1). Three isoforms of PDE3 have been identified in human myocardium (2, 3). They appear to be generated from the PDE3A gene by a combination of alternative transcriptional and post-transcriptional processing, and their amino acid sequences are identical except for the presence of different lengths of N-terminal sequences containing membrane association domains and sites for activation by phosphorylation (Fig. 1). PDE3A-136, present exclusively in microsomal fractions of human myocardium, contains two membrane association domains, “NHR1” and “NHR2,” and three sites for phosphorylation and activation by cAMP-dependent protein kinase and protein kinase B. PDE3A-118, present in microsomal and cytosolic fractions of human myocardium, lacks NHR1 and the most upstream phosphorylation site, whereas PDE3A-94, also present in microsomal and cytosolic fractions of human myocardium, lacks NHR1, NHR2, and all three phosphorylation sites.

The existence of these isoforms is likely to be important with respect to the intracellular compartmentation of cAMP metabolism. In cardiac myocytes, cAMP content is regulated differentially in intracellular compartments represented in microsomal and cytosolic fractions. Changes in cAMP content in these compartments correlate with changes in the phosphorylation of different substrates of cAMP-dependent protein kinase and with different physiologic responses (4–10). In cardiac myocytes from rat hearts, different cyclic nucleotide phosphodiesterases have been shown to regulate cAMP-mediated signaling in spatially and functionally distinct intracellular compartments (11, 12). The fact that there are differences among PDE3 isoforms in human myocardium with respect to intracellular localization domains suggests that these isoforms may be involved in this compartmentation in this tissue. Furthermore, the N-terminal sequence differences among these isoforms might be expected to affect their catalytic activity and inhibitor sensitivity, as occurs with isoforms in the PDE4 family (13, 14). Any such differences are likely to be pertinent to the inotropic actions of PDE3 inhibitors, because these drugs affect cAMP hydrolysis in microsomal and cytosolic fractions of cardiac myocytes with different potencies (15, 16).

These considerations may also have some influence on cGMP-mediated signaling in cardiac muscle. The cAMP hydrolytic activity of PDE3 is inhibited competitively by cGMP, and inhibition of cAMP hydrolysis by cGMP has been shown to contribute to several actions of cGMP, including the stimulation of renin secretion, the potentiation of vasodilatory responses to adrenomedullin, the inhibition of tumor necrosis factor-α-induced NF-kB-dependent inflammatory responses in vascular smooth muscle cells, and the potentiation of delayed rectifier K+ currents in sino-atrial cells (17–20). cGMP can raise intracellular cAMP content in cardiac myocytes by inhibiting PDE3 activity (21), and this may contribute to the potentiation of L-type Ca2+ currents in cardiac myocytes by cGMP-raising agents (22–25). Differences in the sensitivities of PDE3 isoforms in different intracellular compartments to inhibition by cGMP might therefore be important in cGMP-mediated signaling in cardiac myocytes.

Our goal was to gain insight into the possible role of PDE3 isoforms in

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the compartmental regulation of cAMP metabolism in human myocardium. We characterized the catalytic activity and inhibitor sensitivity of these isoforms, and we examined their contribution to total and to cGMP-regulated cAMP hydrolytic activity in cytosolic and microsomal fractions of human myocardium.

MATERIALS AND METHODS

Expression of Recombinant PDE3 Isoforms—Baculovirus-containing constructs of PDE3A-136, PDE3A-118, and PDE3A-94 with the FLAG epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Lys) inserted immediately upstream of the stop codon were prepared as described previously (3, 26). Virus pools were prepared by infecting 4.5 × 10⁶ Sf9 cells suspended in 450 ml of SF-900 II SFM medium (Invitrogen). Unless otherwise stated, all steps were carried out at 27 °C. When cell death reached 50%, cell suspensions were sedimented at 3,000 rpm (JA-20; Beckman Instruments, Fullerton, CA), and the supernatant was collected. 20 ml of this supernatant were added to 2.0 × 10⁸ Sf9 cells suspended in 200 ml of SFM medium. When cell death in this population reached 20%, cell suspensions were sedimented at 1,200 rpm (JA-20; Beckman Instruments) for 10 min at 4 °C. Pelleted cells were washed twice with ice-cold phosphate-buffered saline, resuspended, and incubated for 10 min in ice-cold buffer containing 50 mM Tris, 150 mM NaCl, and 0.1% Nonidet P-40 (pH 7.4, 4 °C). The supernatant fractions were used for the experiments described below.

Preparation of Subcellular Fractions of Human Myocardium—Human left ventricular myocardium was obtained from the explanted hearts of patients with dilated cardiomyopathy undergoing cardiac transplant. Cytosolic and KCl-washed microsomal fractions were prepared by homogenization and differential sedimentation as described previously (3). Each preparation was made from tissue from at least three different hearts.

Immunoprecipitation of PDE3 from Subcellular Fractions—Undiluted rabbit polyclonal antibody (3 μl) raised against the C terminus of human PDE3A (2) was incubated with 30 μl of packed G-Sepharose beads (Amersham Biosciences) for 3 h at 4 °C. Beads with bound antibody were sedimented at 4,000 rpm for 3 min in a Hermle Z180m centrifuge (Labnet, Edison, NJ) and washed with ice-cold buffer containing 20 mM Tris, 150 mM NaCl, and 0.1% Nonidet P-40 (pH 7.4, 4 °C). This process was repeated three times. Cytosolic fractions containing 50 pmol/min of cAMP hydrolytic activity (measured at 0.1 μM cAMP as described below) were added to the antibody/G-Sepharose beads and co-incubated overnight at 4 °C. Beads were removed by sedimentation at 14,000 rpm for 4 min at 4 °C.

Western Blotting—Lysates of Sf9 cells were dissolved in SDS buffer, subjected to SDS-PAGE (10% acrylamide), and transferred electro- phoretically to polyvinylidene fluoride membranes (2). PDE3 was visualized with horseradish peroxidase-conjugated anti-FLAG monoclonal antibody M2 (Sigma) and ECL reagent (Amersham Biosciences).

Quantitation of rtPDE3 Isoforms—C-terminal FLAG-tagged rtPDE3 protein was quantified by enzyme-linked immunosorbent assay. Varying amounts of Sf9 cell lysates were incubated overnight in wells of Nunc Immuno-polystyrene plates with Maxisorb surfaces (VWR Scientific, Denver, CO) at 4 °C. After blocking with 50 μg/ml bovine serum albumin in Tris-buffered saline, wells were incubated with horseradish peroxidase-conjugated anti-FLAG monoclonal antibody M2. ABTS (2,2′-azino-bis(3-ethylbenz-thiazoline-6-sulfonic acid) diaminonitrobenzene) reagent (Sigma) was added at 0.22 mg/ml, and color development (millioptical density/min) was monitored over a 20-min time course.

C-terminal FLAG-tagged bacterial alkaline phosphatase (Sigma) was used as a standard. For all determinations, calculations of the amount of FLAG-tagged protein were based on readings in the range through which the rate of color development varied linearly with the amount of protein per well.

Quantitation of Cyclic Nucleotide Phosphodiesterase Activity and Its Inhibition—Cyclic nucleotide hydrolytic activity in Sf9 cell lysates was quantified at 30 °C by using the two-step snake venom method with [3H]cAMP and [3H]cGMP as substrates (2). EGTA, CaCl₂, and calmodulin (provided by Donald Blumenthal, University of Utah) were included as indicated in the text. Kᵦ values for cAMP and cGMP hydrolytic activity and Kᵦ values for inhibition of cAMP hydrolytic activity by the competitive inhibitor cilostazol were calculated by nonlinear regression using the equation v/Vₘₐₓ = [S]/(Kₛ₊[(inhibitor)/Kᵦ] + [S]). Values for Kᵦ were calculated using the equation Kᵦ = Vₘₐₓ/[PDE3].

PDE3 activity in subcellular fractions of human myocardium was quantified by measuring cAMP hydrolysis as described above in the absence and presence of cilostazol. To minimize possible inhibition of other subfamilies of PDE3 at maximally inhibitory concentrations of cilostazol, these measurements were made at concentrations of cilostazol that inhibited cAMP hydrolytic activity by rtPDE3A submaximally; PDE3 activity was calculated by dividing the amount of activity inhibited by cilostazol in subcellular fractions by the fractional inhibition of rtPDE3 activity at the same cilostazol concentration.

RESULTS

Catalytic Activity and Inhibitor Sensitivity of PDE3 Isoforms—Our first objective was to determine whether there were differences among the three PDE3A isoforms in human myocardium with respect to catalytic activity. To do this, we characterized the catalytic activity of FLAG-tagged recombinant forms of PDE3A-136, PDE3A-118, and PDE3A-94.

FIGURE 1. PDE3 isoforms in human myocardium. Diagram shows N-terminal hydrophobic regions (NHR1 and NHR2) involved in intracellular localization, conserved C-terminal region (CCR) involved in catalytic activity, and sites of phosphorylation by cAMP-dependent protein kinase and protein kinase B (P1, P2, and P3).
PDE3 in Human Myocardium

The apparent molecular weights of the expressed proteins were consistent with published values (3), and there was no evidence of proteolysis of any of the isoforms (Fig. 2). There were no significant differences among the three PDE3 isoforms with respect to $K_m$ for cAMP (TABLE ONE). Our quantitation of rtPDE3 isoforms by enzyme-linked immunosorbent assay allowed a comparison of $k_{cat}$ values for these different isoforms, and we found no significant differences among them in this regard. The variance among preparations with respect to values for $k_{cat}$ was large, however, and the possibility of small differences among the isoforms with respect to $k_{cat}$ cannot be excluded.

We proceeded to examine the sensitivities of the cAMP hydrolytic activity of these isoforms to the competitive inhibitors cGMP and cilostazol. Because cGMP and cAMP are competitive substrates for PDE3, the sensitivity of its cAMP hydrolytic activity to inhibition by cGMP is reflected in the $K_m$ value for cGMP. There were no significant differences among the three PDE3A isoforms in this regard (TABLE TWO). In the case of cilostazol, which is not a substrate, we measured the $K_i$ values for the inhibition of cAMP hydrolysis. As with cGMP, all three PDE3A isoforms were comparable in their sensitivity to inhibition by cilostazol (TABLE TWO). Similar results were obtained for inhibition by the competitive inhibitor milrinone (data not shown).

**TABLE ONE**

| Isoform  | $K_m$, cAMP (nM) | $k_{cat}$, cAMP (min$^{-1}$) |
|----------|------------------|-------------------------------|
| PDE3A-136| 88 ± 11          | 29 ± 11                       |
| PDE3A-118| 93 ± 16          | 30 ± 18                       |
| PDE3A-94 | 79 ± 21          | 31 ± 7                        |

**TABLE TWO**

| Isoform  | $K_m$, cGMP (nM) | $K_i$, cilostazol (nM) |
|----------|------------------|------------------------|
| rtPDE3A-136| 30 ± 5           | 50 ± 10                |
| rtPDE3A-118| 20 ± 2           | 39 ± 11                |
| rtPDE3A-94 | 28 ± 3           | 56 ± 6                 |

We first quantified the effect of [cAMP] and Ca$^{2+}$/calmodulin on the activity of PDE3 itself. As expected (Fig. 3), the activity of rtPDE3A-136 increased when [cAMP] was raised from 0.1 to 1.0 μM. Ca$^{2+}$/calmodulin affected neither the activity of rtPDE3A nor its sensitivity to inhibition by cilostazol or cGMP.

We proceeded to measure cAMP hydrolytic activities in subcellular fractions of human myocardium. At 0.1 μM cAMP, PDE3 comprised the majority of cAMP hydrolytic activity in microsomal fractions in the absence and in the presence of Ca$^{2+}$/calmodulin (Fig. 4). At 1.0 μM cAMP, PDE3 activity increased, but its relative contribution to total cAMP activity was reduced to a small degree by a slightly greater increase in the activity of other (cilostazol-insensitive) cAMP phosphodiesterases.

Results in cytosolic fractions were quite different. At 0.1 μM cAMP, PDE3 comprised the majority of cAMP hydrolytic activity in the absence of Ca$^{2+}$/calmodulin (Fig. 4). In the presence of Ca$^{2+}$/calmodulin, however, the relative contribution of PDE3 to cAMP hydrolytic activity was markedly reduced by a large increase in the activity of cilostazol-insensitive, Ca$^{2+}$/calmodulin-activated cAMP phosphodiesterases (presumably PDE1). As in microsomal fractions, raising the cAMP concentration from 0.1 to 1.0 μM increased the activity of PDE3 in cytosolic fractions, but its relative contribution to total cAMP hydrolytic activity declined because of a larger increase in the activity of cilostazol-insensitive cAMP phosphodiesterases. The decrease in the relative contribution of PDE3 to total cAMP hydrolysis was decreased even further by the addition of Ca$^{2+}$/calmodulin and the consequent increase in the activity of Ca$^{2+}$/calmodulin-activated cAMP phosphodiesterases. At 1.0 μM cAMP, in the presence of Ca$^{2+}$/calmodulin, PDE3 consisted of <20% of the total cAMP hydrolytic activity in cytosolic fractions.

**Effects of cGMP on cAMP Hydrolytic Activity**—Our third objective was to examine the effect of cGMP on cAMP hydrolysis in subcellular fractions. Because cGMP is a competitive inhibitor of PDE3 activity, and because cGMP analogues and agents that raise cGMP content can raise intracellular cAMP content in cardiac myocytes (21, 28–30), our expectation was that cGMP would inhibit cAMP hydrolysis under different conditions to an extent that would reflect the contribution of PDE3 to total cAMP hydrolytic activity.

We first examined the effects of 1.0 μM cGMP on rtPDE3A activity at different concentrations of cAMP in the absence or presence of Ca$^{2+}$/calmodulin. The sensitivity of PDE3A cAMP hydrolytic activity to inhi-
bition by cGMP was not affected by Ca\(^{2+}\)/calmodulin at cAMP concentrations of 0.1 and 1.0 \(\mu M\) (Fig. 5).

We proceeded to quantify the effect of 1.0 \(\mu M\) cGMP on the cAMP hydrolytic activity in subcellular fractions. In microsomal fractions, at both 0.1 and 1.0 \(\mu M\) cAMP, 1.0 \(\mu M\) cGMP inhibited cAMP phosphodiesterase activity (Fig. 6). This inhibition was unaffected by the presence of Ca\(^{2+}\)/calmodulin, and the magnitude of inhibition at 0.1 \(\mu M\) cAMP was comparable with the relative contribution of PDE3 to total activity (Fig. 4). These findings are consistent with the notion that cGMP inhibits cAMP hydrolysis in microsomal fractions by inhibition of PDE3. The somewhat lower magnitude of inhibition seen at 1.0 \(\mu M\) cAMP is consistent with the lower inhibitor:substrate ratio at this concentration.

In contrast, in cytosolic fractions the inhibition of cAMP hydrolysis by cGMP was greater than could be explained on the basis of the contribution of PDE3 to cAMP hydrolytic activity, particularly in the presence of Ca\(^{2+}\)/calmodulin (Figs. 4 and 6). This suggested that cGMP was also inhibiting non-PDE3 cAMP hydrolytic activities in these fractions. To test this, we examined the inhibition of cAMP hydrolysis by cGMP in cytosolic fractions from which PDE3 had been removed by immunoprecipitation with anti-PDE3 antibodies. cGMP had a significant inhibitory effect on non-PDE3 (cilostazol-insensitive) cAMP hydrolytic activity in these fractions (Fig. 7). The fact that a magnitude of this effect was greatly increased in the presence of Ca\(^{2+}\)/calmodulin suggests it reflected inhibition of isoforms in the PDE1 family, which also hydrolyze cAMP and cGMP in a mutually competitive manner (31).

**DISCUSSION**

In previous studies, we identified three isoforms of PDE3 cyclic nucleotide phosphodiesterase localized to different intracellular compartments of cardiac myocytes. Our intent in this study was to gain further insight into the possible roles of these isoforms in the compartment-selective regulation of cAMP-mediated signaling in human myocardium. Our first consideration was whether these isoforms would differ with regard to their catalytic activity and inhibitor sensitivity. In the PDE4 family, as in the PDE3 family, alternative transcriptional and post-transcriptional processing yields isoforms with conserved C-terminal
catalytic domains but different N-terminal domains. Differences in the N-terminal domains of PDE4 isoforms result in major differences in their catalytic activity and inhibitor sensitivity, suggesting that these N-terminal domains have an important role in regulating enzyme activity (13, 14, 32–37). We therefore expected to find significant differences in catalytic activity and inhibitor sensitivity among the three PDE3 isoforms attributable to differences in their N-terminal sequences. Instead, we found that the three PDE3 isoforms are comparable with respect to both $K_m$ and $k_{cat}$ values for cAMP, as well as with respect to their sensitivity to competitive inhibitors of cAMP hydrolysis. (To our knowledge, this is the first comparison of $k_{cat}$ values among PDE3A isoforms.) These observations suggest that the role of the N terminus in PDE3
isoforms is likely to have more to do with intracellular localization than with the allosteric effects on the C-terminal catalytic region. Interactions between the N terminus of PDE4 and other proteins appear to be involved in the intracellular localization of these isoforms (37–40), and previous studies have indicated that the N-terminal hydrophobic domains of PDE3 are involved in intracellular localization in transfected cells at least in part through interactions with other proteins (26, 41). Several proteins that bind to PDE3 isoforms have been identified recently, but their role in the compartmentation of these enzymes remains unknown (42, 43). Experiments designed to identify the mechanisms by which the N-terminal domains regulate the intracellular localization of these isoforms constitute an important direction for future studies.

A second objective was to quantify the contribution of PDE3 isoforms to cyclic nucleotide metabolism in different compartments of human myocardium. We did this by using a method that minimized the effects of PDE3 inhibitors on other cyclic nucleotide phosphodiesterases. We found significant differences in the contribution of PDE3 isoforms to cAMP hydrolytic activity in microsomal and cytosolic fractions of human myocardium that were dependent upon concentrations of cAMP and Ca\(^{2+}\)/calmodulin. Although PDE3 isoforms constitute the major fraction of total cAMP hydrolytic activity in microsomal fractions under all the conditions we examined, their relative contribution to cAMP hydrolytic activity in cytosolic fractions was greatly reduced at higher [cAMP] and in the presence of Ca\(^{2+}\)/calmodulin. This reduction was not because of changes in the activities of the PDE3 isoforms, which increase with increasing [cAMP] and are unaffected by Ca\(^{2+}\)/calmodulin, but to large increases in the activities of PDE1 and other cAMP phosphodiesterase isoforms under these conditions. This suggests that the role of PDE3 in the regulation of cAMP-mediated signaling in intracellular compartments of cardiac myocytes may be highly dependent upon Ca\(^{2+}\) and cAMP concentrations. At higher intracellular [Ca\(^{2+}\)] and [cAMP] (conditions that may occur at higher heart rates or at higher levels of β-adrenergic receptor stimulation), PDE3 isoforms are likely to serve principally to modulate cAMP-mediated signaling selectively in intracellular compartments represented in microsomal fractions as a result of high levels of non-PDE3 cAMP hydrolytic activity in the cytosol. At lower intracellular [Ca\(^{2+}\)] and [cAMP], however, the activities of PDE1 and other cAMP phosphodiesterases in the cytosol are likely to be reduced, increasing the importance of PDE3 in determining cytosolic cAMP content. PDE3 isoforms may regulate cAMP-mediated signaling with much less selectivity for membrane-associated compartments under these conditions. To our knowledge, the presence of high levels of PDE1 activity in cytosolic but not in microsomal fractions of human myocardium has not been documented previously.

If the contribution of PDE3 to compartmental cAMP metabolism is affected by differences in intracellular [Ca\(^{2+}\)] and [cAMP], the effects of PDE3 inhibitors in cardiac myocytes must also depend upon these conditions. This has important implications with regard to the therapeutic actions of PDE3 inhibitors; these agents may have compartment-selective effects on cAMP-mediated signaling in normal myocardium, in which β-adrenergic receptor-mediated cAMP generation is preserved, but this selectivity may be diminished in failing myocardium, in which β-adrenergic receptor-mediated cAMP generation is attenuated, intracellular cAMP content is reduced, and intracellular Ca\(^{2+}\) transients are attenuated (44–47). These considerations may be relevant to the markedly reduced inotropic efficacy of PDE3 inhibitors in failing myocardium and to the adverse effects of chronic treatment with PDE3 inhibitors in patients with dilated cardiomyopathy (16, 48). It should be noted that our results were obtained by using tissue from failing human myocardiun, and the extent to which they apply to normal myocardium is uncertain. Although we and other investigators (49–51) have found no differences between normal and failing human myocardium with respect to PDE3 activity, other investigators (52) have reported recently a decrease in PDE3A1 mRNA expression and PDE3 activity in failing myocardium. The reason for these different observations is unknown. It is also worth considering that PDE1 activity may be altered in failing myocardium and that this might alter the contribution of PDE3 to the regulation of cytosolic cAMP metabolism in this tissue. Although we are unaware of any direct evidence for this, a large increase in PDE1 activity has been noted in mice overexpressing adenyl cyclase (53). A re-examination of the compartmentation of PDE3 isoforms and other cAMP hydrolytic activities in normal and failing human myocardium may be helpful.

Our third objective was to examine the effects of cGMP on cAMP metabolism in intracellular compartments. Several aspects of cGMP-mediated intracellular signaling are mediated through inhibition of cAMP hydrolytic activity (17–20). There is evidence that cGMP raises intracellular cAMP content in cardiac myocytes by inhibiting PDE3 activity (21), and this mechanism may be involved in the potentiation of L-type Ca\(^{2+}\) currents in cardiac myocytes by cGMP-raising agents (22–25). We were therefore interested in determining whether cGMP would have effects on cAMP metabolism in microsomal and cytosolic fractions that reflected the relative contribution of PDE3 to cAMP hydrolysis. We found this to be the case in microsomal fractions. In cytosolic fractions, however, the effects of cGMP on cAMP hydrolysis in cytosolic fractions were greater than could be explained on the basis of PDE3 inhibition alone and involved the additional mechanism of inhibition of cAMP hydrolysis by PDE1. The latter family of enzymes constitutes a large fraction of the cGMP-inhibited cAMP hydrolytic activity in cytosolic fractions of cardiac myocytes but only a minor component of the membrane-associated cGMP-inhibited cAMP hydrolytic activity.

These findings regarding the contributions of PDE1 and PDE3 to cGMP-inhibited cAMP hydrolytic activity in different intracellular compartments of cardiac myocytes are interesting in view of the complex actions of cGMP in these cells. In some studies (54–56), increasing myocardial cGMP content is associated with positive inotropic effects. In other studies (57), no inotropic effects have been observed; and in other studies (58) biphasic effects have been observed. In other studies (59–61), increases in cGMP are associated with negative inotropic effects that can antagonize the actions of cAMP-raising agents. Some of this diversity among observations may have to do with a possible compartmentalization of cGMP metabolism, because of the presence of multiple soluble and membrane-bound forms of guanylate cyclase that are localized and regulated differentially in cardiac myocytes (62, 63). cGMP-mediated signaling has also been shown to be involved in inhibiting pressure- and catecholamine-induced hypertrophy in cardiac myocytes (64–70). Our finding of high levels of PDE1 and PDE3 in cytosolic and microsomal fractions of human myocardium suggests that inhibition of cAMP hydrolysis by cGMP is likely to contribute to cGMP-mediated signaling in this tissue. This has therapeutic relevance because of the common concomitant use of cGMP-raising vasodilators and cAMP-raising inotropic agents in the treatment of heart failure. Further clarification of the interrelationship of cGMP- and cAMP-mediated signaling in human myocardium will be an important area for future studies.

REFERENCES

1. Shakur, Y., Holst, L. S., Landstrom, T. R., Movsesian, M., Degerman, E., and Manganiello, V. (2001) Prog. Nucleic Acids Res. Mol. Biol. 66, 241–277
2. Choi, Y. H., Ekholm, D., Krall, J., Ahmad, F., Degerman, E., Manganiello, V. C., and...
