Subclasses of Cyclic AMP-Specific Phosphodiesterase in Left Ventricular Muscle and Their Involvement in Regulating Myocardial Contractility

Ronald E. Weishaar, Dianne C. Kobylarz-Singer, Robert P. Steffen, and Harvey R. Kaplan

Ventricular muscle contains a low $K_m$, cyclic AMP-specific form of phosphodiesterase (PDE III), which is believed to represent the site of action for several of new cardiotonic agents including imazodan (CI-914), amrinone, cilostamide, and enoximone. However, species differences in the inotropic response to these agents have raised questions about the relationship between PDE III inhibition and cardiotonic activity. The present study demonstrates that these differences can be accounted for by the presence of two subclasses of PDE III in ventricular muscle and variations in the intracellular localization of these two enzymes. For these experiments, PDE III was initially isolated from canine, guinea pig, and rat left ventricular muscle. The results demonstrate that canine left ventricular muscle contains two functional subclasses of PDE III: an imazodan-sensitive form, which is membrane bound, and an imazodan-insensitive form, which is soluble. Although only weakly inhibited by imazodan, this latter enzyme is potently inhibited by the selective PDE III inhibitors, Ro 20-1724 and rolipram. Guinea pig ventricular muscle also contains the imazodan-sensitive subclass of PDE III. Unlike canine left ventricle, however, this enzyme is soluble in the guinea pig. No membrane-bound subclass of PDE III was observed in the guinea pig. Rat left ventricle possesses only the soluble form of PDE III, which apparently represents a mixture of the imazodan-sensitive and imazodan-insensitive subclasses of PDE III. Measurements of in vivo contractility in these three species showed that imazodan exerts a potent positive inotropic effect only in the dog, in which the imazodan-sensitive subclass of PDE III is membrane bound. In addition, a strong correlation was observed between in vitro inhibition of the membrane-bound, imazodan-sensitive PDE III from canine ventricular muscle and the in vivo positive inotropic response to imazodan, amrinone, and related cardiotonic agents in the dog. Inhibitors of the imazodan-insensitive subclass of PDE III did not exert any pronounced inotropic effect in the dog. These results demonstrate that functional subclasses of PDE III exist in ventricular muscle and suggest that species differences in the positive inotropic response to imazodan and related cardiotonics can be attributed to the proper intracellular localization of the imazodan-sensitive subclass of PDE III. (Circulation Research 1987;61:539–547)

During the past decade, a number of new positive inotropic agents have been developed for the treatment of congestive heart failure. Of these, the selective phosphodiesterase inhibitors such as imazodan (CI-914), amrinone, milrinone, and enoximone have received considerable attention. The cardiotonic activity of these agents is normally attributed to their selective inhibitory effect on the low $K_m$, cyclic adenosine monophosphate (cAMP)-specific form of phosphodiesterase (PDE III), which has been identified in ventricular muscle from several species. However, preliminary studies have shown that although these agents exert potent inotropic effects on canine, cat, and rhesus monkey left ventricular muscle, they exert little or no inotropic effect in other species, such as the rat and the hamster. This observation is surprising since PDE III has been identified in ventricular muscle from all of these species.

In a previous report, Weishaar et al. observed that although imazodan, amrinone, and other selective phosphodiesterase inhibitors exert a potent inhibitory effect on the cAMP-specific phosphodiesterase (PDE III) isolated from guinea pig left ventricle and human platelets, these agents exert a biphasic inhibitory effect on PDE III isolated from bovine coronary arteries, suggesting the presence of two subclasses of the enzyme or else an unusual allosteric interaction. In a subsequent study, Kobylarz and coworkers compared the in vitro inhibitory effect of imazodan on PDE III isolated from left ventricular muscle of hamster, rat, guinea pig, and rhesus monkey with the in vivo positive inotropic response to imazodan in these four species. A good correlation was observed for the rhesus monkey, rat, and hamster. In the guinea pig, however, imazodan exerted a potent in vitro inhibitory effect on ventricular PDE III, while exerting only a weak positive inotropic effect when administered in vivo. The present study was undertaken to reconcile these observations. In the course of these experiments, it was discovered that ventricular muscle contains two subclasses of PDE III. Although both enzymes have a comparable affinity for cAMP as a substrate, they differ...
new insights regarding regulation of cAMP metabolism within the cardiac cell. These observations also provide the proper localization of the subclasses of PDE III cAMP-specific phosphodiesterase inhibitors based on the proper localization of the subclasses of PDE III within the cardiac cell. These observations also provide new insights regarding regulation of cAMP metabolism in cardiac muscle.

Materials and Methods

Procedures for Isolating Ventricular Phosphodiesterases

Soluble and membrane-bound phosphodiesterases were discretely isolated using a modification of the isolation procedure described by Elks and Manganiello. This procedure employed milder conditions for tissue homogenization than were used in previous isolation procedures to prevent the loss of membrane-bound enzymes into the soluble fraction. After mincing, the ventricular tissue was homogenized in 10 volumes of a buffer containing 0.25 M sucrose, 10 mM Tris-HCl (pH 7.8), 5 mM MgCl₂, and 0.2 mM EGTA, as described by Elks and Manganiello. The peptidase inhibitors leupeptin, pepstatin A, and phenylmethylsulfonyl fluoride (PMSF) were also included in this homogenizing buffer (final concentration 100 nM each). The mince was homogenized with a Potter-Elvehjem hand-held homogenizer (Thomas Scientific, Swedesboro, N.J.) fitted with a serrated Teflon pestle (4 passes, 10 strokes per pass, with a 30-second rest period between each pass). This and all subsequent procedures were performed at 4°C. The homogenate was then centrifuged at 100,000 g for 40 minutes, and the resulting supernatant was discarded, the pellet was resuspended in 10 volumes of homogenizing buffer containing 0.4 M NaCl, 1% Triton X-100, and 0.1% brij 30 (polyoxyethylene 4-lauryl ether) and incubated overnight at 4°C. The detergent-extracted proteins were then separated by centrifugation at 100,000 g for 40 minutes. Using this procedure, Elks and Manganiello have reported that approximately 85–90% of the phosphodiesterase activity in the pellet is recovered in the supernatant. The detergent-extracted proteins were then dialyzed for 5 hours against 4 liters of 70 mM sodium acetate/5 mM 2-mercaptoethanol (pH 6.5) containing protease inhibitors. The dialyzed proteins were then applied to a DEAE-cellulose anion-exchange column and eluted as described above.

Measuring Phosphodiesterase Activity

Phosphodiesterase activity was measured, as described by Thompson et al., in a reaction medium containing 40 mM Tris-HCl (pH 8.0), 5 mM MgCl₂, and 4 mM 2-mercaptoethanol. Unless otherwise indicated, the concentration of substrate (H-cAMP or H-cGMP) was 1.0 μM. Selective and nonselective phosphodiesterase inhibitors were dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 2.5%. This concentration of DMSO inhibited enzyme activity by approximately 10%. The IC₅₀ values (concentrations that produced 50% inhibition of substrate hydrolysis) for the various agents examined were determined from concentration-response curves in which concentrations typically ranged from 10⁻⁴ to 10⁻⁵ for the more potent inhibitors and 10⁻⁶ to 10⁻⁷ M for the less potent inhibitors (half-log increments).

In Vivo Contractility Measurement

To evaluate species differences in the positive inotropic response to the selective cAMP-specific phosphodiesterase (PDE III) inhibitor imazodan (CI-914), animals were anesthetized with sodium pentobarbital (60 mg/kg i.p. for rats and guinea pigs, 32 mg/kg i.v. for dogs). For the rat and guinea pig, a fluid-filled polyethylene cannula (PE 50) was introduced into the left ventricular chamber via the carotid artery. This cannula was used for measuring left intraventricular pressure and its first derivative (+ dP/dt), which was used as a measure of myocardial contractility. Imazodan was administered in rising dose bolus fashion via a catheter in the jugular vein.

For studies with dogs, the trachea was intubated, and the animals were ventilated artificially with room air by means of a Harvard constant volume positive pressure...
respirator (Harvard Apparatus, South Natick, Mass.). Anesthesia was maintained by continuous infusion of sodium pentobarbital (3.5 mg/kg/hr) via a catheter in the left femoral vein. A Micro-Tip pressure transducer catheter (Millar Instruments, Houston, Tex.) was introduced via the right femoral artery into the thoracic aorta for continuous measurement of arterial blood pressure. A second Millar pressure transducer catheter was advanced from the left carotid artery into the lumen of the left ventricular chamber for continuous measurement of left intraventricular developed pressure and its first derivative ( + dP/dt).

Following surgical preparation, a 30-minute period was allowed for recovery and establishment of stable cardiovascular performance, after which imazodan was administered intravenously at an infusion rate of 1 ml/min with increasing doses ranging from 1–1,000 µg/kg. For all three species, saline was also administered to an additional group of animals that served as a stability control. Dobutamine (1–30 µg/kg, i.v.) was also administered to all three species.

In a subsequent series of experiments, imazodan was also administered to anesthetized hamsters and rhesus monkeys. For these experiments, hamsters were surgically prepared as described previously for rats and guinea pigs, and rhesus monkeys were prepared as described for dogs.

A number of cAMP-specific phosphodiesterase (PDE III) inhibitors were administered to anesthetized dogs to assess the relation between in vitro inhibitory effects on isolated PDE III activity and in vivo changes in left ventricular contractility. The isotropic effect of several of these agents was evaluated in the presence and absence of prior β-adrenergic blockade with either propranolol (0.5 mg/kg) or nadalol (1.0 mg/kg) to separate direct from indirect effects on contractility. These latter agents were administered 10 minutes prior to administration of the selective PDE III inhibitors, and their β-adrenergic antagonist activity was assessed by their ability to block intravenously administered isoproterenol reversibly.

Reagents

All agents used were of the highest obtainable commercial purity. [2,8-3H]-labelled cAMP (30–50 Ci/mmol) and [8-3H(N)]-labelled cGMP (10–25 Ci/mmol) were obtained from New England Nuclear, Boston, Mass. Calmodulin (from bovine heart), Ophiophagus hannah snake venom, theophylline, cAMP, cGMP, pepstatin A, leupeptin, phenylmethylsulfonyl-fluoride, Triton X-100, and brij 30 were obtained from Sigma Chemical Co, St. Louis, Mo. DEAE-cellulose ion exchange resin was obtained from Matheson, Coleman and Bell, East Rutherford, N.J. (product no. CX583-1-9178). Sodium pentobarbital was obtained from Butler Co. Cl-914 (hydrochloride salt), Cl-930 (hydrochloride salt), amrinone, milrinone, enoximone, cilostamid, and Ro 20-1724 were prepared by the Warner-Lambert/Parke Davis Chemistry Department. Rolipram was obtained from Scherring AG Pharmaceutical Company, Berlin. Dobutamine was obtained from the Eli Lilly Company, Indianapolis. The purity of these agents was >99%.

Statistical Evaluation

IC₅₀ values were calculated according to the method of Hubert,¹ as described previously.¹¹ The application of this method to the analysis of concentration/dose-response relations has been described in detail by Waud.¹⁶ Kₘ (substrate concentration at which half-maximal reaction velocity is observed) and Vₘₐₓ (maximal velocity of the reaction) values were determined using the method of Hofstee.¹⁷

Results

Isolation of Multiple Forms of Phosphodiesterase

The different forms of phosphodiesterase (PDE) present in canine, guinea pig, and rat left ventricular muscle were isolated, and their intracellular localization (soluble versus membrane bound) was characterized. Figure 1 is a representative chromatograph demonstrating the isolation of the different phosphodiesterases present in canine ventricular muscle. As can be seen, three soluble forms of phosphodiesterase were identified, which were labelled PDE I, PDE II, and PDE III based on their order of elution from DEAE-cellulose. PDE I hydrolyzes cAMP and cGMP with equal affinity (Kₘ = 1.0 µM for both substrates), and its activity can be stimulated severalfold by calmodulin (data not shown). PDE II also hydrolyzes cAMP and cGMP with equal affinity (Kₘ = 20–30 µM for both substrates) and is insensitive to calmodulin stimulation. The cAMP hydrolytic activity of PDE II, however, can be stimulated by low concentrations of cGMP (data not shown). PDE III preferentially hydrolyzes cAMP (Kₘ = 1.0 µM) and is insensitive to calmodulin stimulation (Figure 1). Canine left ventricular muscle also contains a membrane-bound, low Kₘ, cAMP-specific phosphodiesterase (membrane-bound PDE III), which is insensitive to calmodulin stimulation.

Guinea pig and rat left ventricle also possess three soluble forms of phosphodiesterase, which elute in the same regions as the canine soluble phosphodiesterases (chromatographs not shown). The cyclic nucleotide hydrolytic properties of these three enzymes are similar to the soluble phosphodiesterases present in canine left ventricle, with the exception that PDE I in the rat displayed a clear preference for cGMP as substrate. In contrast to canine left ventricular muscle, no membrane-bound phosphodiesterases were identified in ventricular muscle from guinea pigs or rats.

Characterization of Soluble and Membrane-Bound cAMP-Specific Phosphodiesterase

Although the membrane-bound cAMP-specific phosphodiesterase (PDE III) present in canine ventricular muscle and the soluble PDE III present in ventricular muscle from all three species were similar kinetically (comparable Kₘ and Vₘₐₓ values) and also eluted from DEAE-cellulose at comparable concentrations of sodium acetate, significant differences were observed when the response of these enzymes to various selective
PDE III inhibitors was examined. As Figure 2 (top panels) illustrates, the activity of the canine membrane-bound PDE III is potently inhibited by the selective PDE III inhibitor imazodan (CI-914), while the selective PDE III inhibitor rolipram exerts only a weak inhibitory effect on this enzyme. In contrast, the activity of the canine soluble PDE III is only weakly inhibited by imazodan but is potently inhibited by the selective PDE III inhibitor rolipram. The soluble PDE III isolated from guinea pig left ventricle appears to be comparable to the membrane-bound PDE III isolated from canine left ventricle since it is potently inhibited by imazodan and is only weakly inhibited by rolipram (Figure 2, middle panels). Rat left ventricle possess only the soluble form of PDE III. This enzyme is inhibited to the same extent by both imazodan and rolipram, which suggests that in the rat, both the imazodan-sensitive subclass of PDE III and the imazodan-insensitive subclass are present in the soluble fraction (Figure 2, bottom panels). This possibility is supported by the fact that the concentration-response curves obtained with both inhibitors are biphasic, suggesting the presence of two enzymes.

The inhibitory effects of a variety of selective and nonselective phosphodiesterase inhibitors on the soluble and membrane-bound cAMP-specific phosphodiesterases present in ventricular muscle of all three species are summarized in Table 1.

Based on differences in the response to selective PDE III inhibitors such as imazodan and rolipram, these results indicate that functional subclasses of the cAMP-specific phosphodiesterase (PDE III) exist in ventricular muscle. These results also suggest that, at least for the imazodan-sensitive subclass of cAMP-specific phosphodiesterase, the same subclass of PDE III can exist in both soluble and membrane-bound forms.

Subclasses of cAMP-Specific Phosphodiesterase and Ventricular Contractility

To assess the involvement of the different subclasses of cAMP-specific phosphodiesterase (PDE III) in regulating ventricular contractility, the in vivo positive inotropic response to several selective PDE III inhibitors was evaluated in the anesthetized dog. As Table 2 illustrates, the selective canine membrane-bound PDE III inhibitors imazodan, CI-930 (the methyl analogue of imazodan), and amrinone all exert a potent positive inotropic effect in the anesthetized dog. Although prior administration of the β-receptor blocking agents propranolol or nadolol reduced the positive inotropic response to the selective inhibitors of the membrane-bound, imazodan-sensitive subclass of PDE III, these agents still exert significant effects on contractility following β-receptor blockade (Table 2). However, prior administration of β-receptor blocking agents virtually abolished the positive inotropic response to selective inhibitors of the soluble, imazodan-
insensitive subclass of PDE III, Ro 20-1724 and rolipram, indicating that these agents did not exert any appreciable direct inotropic effect in the dog. Possible explanations for the initial positive inotropic response to rolipram and Ro 20-1724 will be addressed in the "Discussion."

Figure 3 also demonstrates that there is significant correlation between the in vivo positive inotropic response to a number of selective PDE III inhibitors and their in vitro inhibitory effects on membrane-bound PDE III isolated from canine left ventricle ($r = 0.985$).

To assess the relation between intracellular location of the imazodan-sensitive subclass of PDE III and ventricular contractility, the in vivo positive inotropic

**Figure 2.** Inhibitory effects of imazodan (left panel) and rolipram (right panel) on the activity of the soluble cAMP-specific phosphodiesterase ($\Delta$) and the membrane-bound cAMP-specific phosphodiesterase (■) isolated from canine, guinea pig, and rat left ventricle. Enzymes were isolated as described in the "Materials and Methods." Each symbol represents the mean of 2-4 separate duplicate determinations of percent inhibition of PDE III hydrolytic activity (standard error bars omitted for clarity; error bars are generally contained within the symbol). Substrate concentration for these experiments was 1.0 $\mu$M cAMP.

**Table 1. Effect of Selective and Nonselective Phosphodiesterase Inhibitors on Subclasses of cAMP-Specific Phosphodiesterase (PDE III) in Canine, Guinea Pig, and Rat Left Ventricular Muscle**

<table>
<thead>
<tr>
<th>Agent</th>
<th>IC$_{50}$ (µM)</th>
<th>Dog</th>
<th>Guinea pig</th>
<th>Rat</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soluble Membrane bound</td>
<td>Soluble Membrane bound</td>
<td>Soluble Membrane bound</td>
<td></td>
</tr>
<tr>
<td>Imazodan (CI-914)</td>
<td>180 2.6</td>
<td>1.3</td>
<td>—*</td>
<td>—20</td>
</tr>
<tr>
<td>Cilostamide</td>
<td>31 0.02</td>
<td>0.19</td>
<td>—*</td>
<td>—3.0</td>
</tr>
<tr>
<td>cGMP</td>
<td>1,500 0.44</td>
<td>2.2</td>
<td>—*</td>
<td>—8.0</td>
</tr>
<tr>
<td>Theophylline</td>
<td>310 280</td>
<td>330</td>
<td>—*</td>
<td>380</td>
</tr>
<tr>
<td>Rolipram</td>
<td>1.0 360</td>
<td>&gt;100</td>
<td>—*</td>
<td>—20</td>
</tr>
<tr>
<td>Ro 20-1724</td>
<td>18 170</td>
<td>220</td>
<td>—*</td>
<td>—80</td>
</tr>
</tbody>
</table>

IC$_{50}$ values (concentration that inhibits substrate hydrolysis by 50%) were determined from concentration-response curves, which concentrations typically ranged from 10$^{-8}$ to 10$^{-3}$ M for the more potent inhibitors and from 10$^{-3}$ to 10$^{-1}$ M for the less potent inhibitors (half-log increments). Enzyme activity was measured as described in "Materials and Methods." Two to four concentration-response curves were generated for each agent, typically using different enzyme preparations for each concentration-response. Since biphasic responses were obtained when the inhibitory effect of the selective PDE III inhibitors on rat soluble PDE III were examined, approximate IC$_{50}$ values are given.

*Guinea pig and rat ventricular muscle do not possess a membrane-bound cAMP-specific phosphodiesterase.
Bound and Soluble cAMP-Specific Phosphodiesterase (PDE III) Inhibitors in Anesthetized Dog

Rolipram
Ro 20-1724
Amrinone
CI-930

Imazodan (CI-914)

Agent

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Table 2. Positive Inotropic Response to Selective Membrane-Bound and Soluble cAMP-Specific Phosphodiesterase (PDE III) Inhibitors in Anesthetized Dog

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (mg/kg i.v.)</th>
<th>Percent increase in left ventricular +dP/dT* Before β-blockade</th>
<th>After β-blockade†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imaizodan (CI-914)</td>
<td>0.31 (5)</td>
<td>80 ± 11†</td>
<td>62 ± 9‡</td>
</tr>
<tr>
<td>CI-930</td>
<td>0.031 (5)</td>
<td>76 ± 9†</td>
<td>47 ± 8‡</td>
</tr>
<tr>
<td>Amrinone</td>
<td>1.0 (8)</td>
<td>61 ± 6‡</td>
<td>40 ± 4†</td>
</tr>
<tr>
<td>Ro 20-1724</td>
<td>0.10 (3)</td>
<td>108 ± 7‡</td>
<td>16 ± 5**</td>
</tr>
<tr>
<td>Rolipram</td>
<td>0.01 (3)</td>
<td>75 ± 13‡</td>
<td>8 ± 3**</td>
</tr>
</tbody>
</table>

*Values represent the mean ± SEM of the number of separate experiments in parentheses. Doses were chosen to produce comparable inotropic responses in the absence of β-adrenergic blockade.

†Each dog was used as its own control to calculate percentage increases in + dP/dt. +0.5 mg/kg i.v. propranolol or 1.0 mg/kg i.v. nadolol were used to block β-receptors. Both agents reversibly abolished the positive inotropic response to isoproterenol without producing excessive cardiac depression. Significantly different from predrug value (p<0.05). **Significantly reduced from value obtained prior to β-blockade (p<0.05).

response to imazodan was evaluated in the anesthetized dog, rat, and guinea pig. These three species were chosen since they vary as to the intracellular location of the imazodan-sensitive subclass of PDE III (membrane bound in the dog and soluble in the guinea pig and the rat). As Figure 4A shows, imazodan exerts a potent positive inotropic response in the anesthetized dog, while exerting only a weak positive inotropic response in the anesthetized guinea pig. In the anesthetized rat, no positive inotropic response to imazodan was observed. Intravenous administration of the β-receptor stimulant dobutamine demonstrated that comparable increases in left ventricular contractility could be produced in all three species (Figure 4B). On the basis of these observations, it appears that the intracellular location of the imazodan-sensitive subclass of PDE III is an important factor in determining the degree to which this enzyme contributes to the regulation of ventricular contractility.

To confirm this hypothesis, two additional species, the rhesus monkey and the hamster, were also studied. These two species were chosen because they vary considerably in their inotropic response to the selective PDE III inhibitor imazodan. A membrane-bound cAMP-specific was identified in rhesus monkey left ventricular muscle, and the properties of this enzyme were comparable to those previously described for the membrane-bound cAMP-specific phosphodiesterase present in canine ventricular muscle (potently inhibited by imazodan and cGMP and weakly inhibited by rolipram and Ro 20-1724). As Table 3 shows, imazodan exerts a similar inhibitory effect on the membrane-bound, cAMP-specific phosphodiesterase (PDE III) isolated from canine and rhesus monkey left ventricle and also evokes a comparable inotropic response in both species.

No membrane-bound imazodan-sensitive PDE III was identified in hamster left ventricular muscle (Table 3). As with other species in which this enzyme is not membrane bound, e.g., guinea pig and rat, imazodan evoked only a weak inotropic response when administered in vivo to the hamster.

The relation between intracellular location of the

Table 3. Relation Between Presence of Membrane-Bound Imazodan-Sensitive cAMP-Specific Phosphodiesterase and Positive Inotropic Response to Imazodan in 5 Species

<table>
<thead>
<tr>
<th>Species</th>
<th>Inhibition of membrane-bound cAMP-specific phosphodiesterase by imazodan (IC50, μM)</th>
<th>Positive inotropic response to imazodan (EC50, μg/kg i.v.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>2.6</td>
<td>0.018</td>
</tr>
<tr>
<td>Rhesus monkey</td>
<td>10.5</td>
<td>0.007</td>
</tr>
<tr>
<td>Rat</td>
<td>—*</td>
<td>—†</td>
</tr>
<tr>
<td>Hamster</td>
<td>—*</td>
<td>3.000</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>—*</td>
<td>0.300</td>
</tr>
</tbody>
</table>

IC50 values (concentration that inhibits substrate hydrolysis by 50%) were determined from concentration-response curves, in which concentration ranged from 10^-8 to 10^-2 M for the more potent inhibitors and 10^-5 to 10^-3 M for the less potent inhibitors (half-log increments). Enzyme activity was measured as described in "Materials and Methods." Two to four concentration-response curves were generated for dog and rhesus monkey membrane-bound cAMP-specific phosphodiesterase. EC50 values represent the dose of imazodan required to increase +dP/dt 20% above basal levels. Imazodan was administered to 3 to 6 animals from each species.

*No membrane-bound, imazodan-sensitive subclass of PDE III was present in rat, hamster, or guinea pig left ventricular muscle.
†Imazodan did not exert a positive inotropic effect in the rat.

Figure 3. Correlation between the in vitro inhibitory effect of various selective PDE III inhibitors on the membrane-bound cAMP-specific phosphodiesterase present in canine ventricular muscle and the in vivo positive inotropic response to these agents in the anesthetized dog. Ventricular contractility was measured as described in "Materials and Methods." IC50 (concentration that inhibits substrate hydrolysis by 50%) values were determined from 2-4 separate duplicate determinations of percent inhibition of PDE III hydrolytic activity. EC50 (dose that increases basal contractility by 50%) values were determined from measuring dose-dependent increases in contractility in 4-6 anesthetized dogs.
imazodan-sensitive and imazodan-insensitive subclasses of PDE III and regulation of myocardial contractility is illustrated in Figure 5.

Discussion

The relation between cAMP and myocardial contractility has been well documented, and agents that increase the synthesis of cAMP, e.g., β-receptor stimulants, as well as agents that inhibit the degradation of cAMP such as phosphodiesterase inhibitors, all increase myocardial contractility. Multiple forms of phosphodiesterase have recently been identified in ventricular muscle, and although nonselective inhibitors of these different enzymes, such as theophylline and papaverine, increase myocardial contractility in several animal models, there are profound species differences in the positive inotropic response to selective inhibitors of the cAMP-specific form of phosphodiesterase (PDE III) such as imazodan (CI-914) and amrinone. The purpose of the present study was to elucidate the basis for these species differences in the positive inotropic response to the selective PDE III inhibitors such as imazodan, thereby resolving the issue of the contribution that inhibition of PDE III makes to the cardiotonic response to these agents. During the course of this investigation, subclasses of PDE III were identified in ventricular muscle, and their involvement in modulating ventricular contractility was determined.

For this study, the cAMP-specific phosphodiesterase (PDE III) was isolated from left ventricular muscle of the dog, rat, and guinea pig. These enzymes were similar in many respects, including 1) equal insensitivity to calmodulin, 2) similar $K_m$ and $V_{max}$ values for cAMP, 3) elution from DEAE-cellulose at comparable concentrations of sodium acetate, and 4) equal inhibition by the nonselective phosphodiesterase inhibitor theophylline. Nevertheless, major differences were observed with regard to intracellular localization (soluble versus membrane bound), and the response to various selective PDE III inhibitors. Based on the latter difference, ventricular PDE III can be divided into two functional subclasses: one that is sensitive to inhibition by the selective PDE III inhibitors imazodan, cGMP, and cilostamide (and insensitive to the selective PDE III inhibitors Ro 20-1724 and rolipram) and one that is sensitive to inhibition by Ro 20-1724 and rolipram (and insensitive to inhibition by imazodan, cGMP, and cilostamide).

The identification of subclasses of cAMP-specific phosphodiesterases (PDE III) in ventricular muscle, as well as the availability of selective inhibitors of each
subclass, makes possible experiments aimed at clarifying the role each subclass plays in regulating myocardial contractility. Thus, of the various selective PDE III inhibitors administered to anesthetized dogs, only imazodan, CI-930 (the methyl analogue of imazodan), and amrinone exerted a direct positive inotropic effect. The involvement of the imazodan-sensitive subclass of PDE III with regulation of myocardial contractility was further supported by the observation that a significant correlation exists between the inhibitory effects that imazodan, amrinone, and several other cardiotonic agents exert on this subclass of PDE III and their positive inotropic effects in the anesthetized dog. Although the selective PDE III inhibitors Ro 20-1724 and rolipram also increased myocardial contractility, their positive inotropic effect could be virtually abolished by prior β-receptor blockade, indicating that neither agent exerted a significant direct effect on ventricular contractility. One possible explanation for the inotropic activity of these latter agents prior to β-receptor blockade could be a reflex stimulation of the heart in response to a reduction in peripheral resistance. A reduction in peripheral resistance has previously been described for the selective PDE III inhibitor imazodan. However, in the present study, no reduction in peripheral resistance was observed with either rolipram or Ro 20-1724. Indeed, a slight increase in resistance was observed with both agents. An alternative explanation for the inotropic activity prior to β-blockade could be an enhancement of norepinephrine release from presynaptic nerve terminals. Wachtele has previously demonstrated that both Ro 20-1724 and rolipram increase norepinephrine turnover and also potentiate postsynaptic noradrenergic transmission.

The finding that the imazodan-sensitive subclass of PDE III can exist in either a membrane-bound form (in dog ventricle) or in a soluble form (in guinea pig and rat left ventricle) also allowed for an examination of the role intracellular localization of this subclass of PDE III plays in the regulation of myocardial contractility. Thus, the observation that imazodan exerts a potent positive inotropic effect in the anesthetized dog, while exerting little or no inotropic effect in the guinea pig and the rat, suggests that the membrane-bound form of the imazodan-sensitive subclass of PDE III exerts a much greater influence on ventricular contractility than does the soluble form. In addition, the observation that intravenous administration of the β-receptor stimulant dobutamine produced comparable increases in left ventricular contractility in all three species suggests that the differences observed in response to the imazodan are not due to intrinsic differences in contractile function. Subsequent studies in the rhesus monkey (in which the membrane-bound imazodan-sensitive subclass of PDE III is present) and the hamster (in which this enzyme is absent) support this hypothesis. Although the results of the present study do not eliminate differences in other factors that influence contractility, e.g., afterload and heart rate, as contributors to the variations in the inotropic response to imazodan, the finding of a clear difference in the intracellular localization of the presumed target enzyme for imazodan and amrinone strongly suggests that this is an important factor in modulating the involvement of these agents with myocardial contractility.

Kaufman and coworkers have recently provided evidence to suggest that in the dog, the membrane-bound subclass of PDE III is associated with the sarcoplasmic reticulum but not with the mitochondria, the sarcolemma, or the contractile proteins. Such specificity provides further support for the argument that the intracellular localization of the imazodan-sensitive subclass of PDE III is an important factor in mediating the positive inotropic response to selective inhibitors of the enzyme. Localization of this subclass of PDE III in other compartments within the cardiac cell, e.g., the cytoplasm, as is observed in the guinea pig, may have the effect of partially "uncoupling" the enzyme in terms of its ability to regulate contractility. Such uncoupling would mean that the increases in cAMP that result from inhibition of PDE III would produce lesser increases in contractility than would otherwise occur since such increases would not be in the most appropriate compartments within the cardiac cell.

Subclasses of cAMP-specific phosphodiesterase (PDE III) have previously been identified in several other tissues and cells. Yamamoto et al have described such subclasses in calf liver and have shown that they are also differentially inhibited by cGMP and various selective PDE III inhibitors such as cilostamide and Ro 20-1724. Recently, Elks and Manganiello showed that the cilostamide-sensitive subclass of PDE III may be more important in regulating lipolysis in 3T3-L1 adipocytes than the Ro 20-1724-sensitive subclass of PDE III. These authors have also reported that both the nonselective phosphodiesterase inhibitor isobutyl-methylxanthine and the selective PDE III inhibitor Ro 20-1724 enhance cellular differentiation, whereas the selective PDE III inhibitor cilostamide had no effect on differentiation. These latter results suggest a discrete role for the Ro 20-1724-sensitive subclass of PDE III in regulating differentiation.

In summary, the present study demonstrates that functional subclasses of the cAMP-specific phosphodiesterase (PDE III) exist in ventricular muscle and also that the imazodan-sensitive subclass of PDE III is important in modulating the in vivo positive inotropic response to imazodan, amrinone, and several other novel cardiotonics. The results also demonstrate that the species differences that exist in the cardiotonic response to imazodan and amrinone can be accounted for by differences in the intracellular localization of the imazodan-sensitive subclass of PDE III. Taken collectively, these observations provide further support for the hypothesis that the cardiotonic response to imazodan and amrinone is due to their inhibitory effect on the cAMP-specific phosphodiesterase, and they also provide important new insights into the relation between cAMP and myocardial contractility.
Acknowledgments

The authors wish to express their appreciation to Drs. Sandra Burke and Ila Suicar and to George Dodd, Ronald Potoczak, Robert McNish, and Denise Bouche for their assistance.

References

27. Krop S: The influences of "heart stimulants" on the contraction of isolated mammalian cardiac muscle. J Pharmacol Exp Ther 1942;82:48–62
32. Elks ML, Manganiello VC: Selective effect of phosphodiesterase inhibitors on different phosphodiesterases, adenosine 3',5'-monophosphate metabolism, and lipolysis in 3T3-L1 adipocytes. Endocrinology 1984;115:1262–1268

KEY WORDS • ventricular muscle • phosphodiesterase • contractility • cardiotonic agents • phosphorylation • cAMP
Subclasses of cyclic AMP-specific phosphodiesterase in left ventricular muscle and their involvement in regulating myocardial contractility.
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Circ Res. 1987;61:539-547
doi: 10.1161/01.RES.61.4.539

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/61/4/539

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