Pharmacology of positive inotropic phosphodiesterase III inhibitors

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Cardiac phosphodiesterase III (PDE) inhibitors derived from pyridinone, imidazolone, pyridazinone and related structures form a new class of positive inotropic vasodilator agents (e.g. milrinone) that are beneficial in the treatment of acute and chronic heart failure. These agents inhibit the intracellular hydrolysis of cyclic AMP, thereby promoting cyclic AMP-catalysed phosphorylation of sarcolemmal calcium channels and activating the calcium pump. Drugs such as milrinone have a wider therapeutic index than the cardiac glycosides. They also have vasodilator and lusitropic actions and are devoid of the central stimulant actions that narrow the therapeutic index of theophylline and other methylxanthines. Receptor down-regulation, which curtails the inotropic efficacy of beta-adrenoceptor agonists, does not compromise the efficacy of PDE inhibitors. The effectiveness of these new agents is, however, dependent upon some degree of basal adenylate cyclase activity. Individual PDE inhibitors differ in terms of both chronotropic and extracardiac properties. The reasons for this are not yet fully understood.

Biochemical mechanism of action

Ten years ago, Farah and Alousi[1] described a synthetic positive inotropic vasodilator agent, amrinone, that is structurally unrelated to cardiotonic steroids, catecholamines or methylxanthines. The cellular mechanism of the inotropic action of amrinone was shown not to involve inhibition of Na, K-ATPase (the receptor for cardiotonic steroids) or beta-adrenoceptors[2]. Despite its different chemical structure, amrinone was found to share inotropic, electrophysiological and phosphodiesterase (PDE) inhibiting properties with the classical methylxanthine PDE inhibitor theophylline, leading to its designation as a positive inotropic PDE inhibitor[3-4]. Subsequent refined studies with purified cardiac PDE isoforms revealed selective inhibition of only one of the three cardiac isoforms, the cAMP-specific PDE III, by amrinone as opposed to the non-selective inhibition of these isoforms exerted by theophylline[5]. This view has recently been challenged, however, by the finding that milrinone and other amrinone-like agents also inhibit cardiac PDE II, provided that the partial proteolysis of this isozyme (associated with purification) is prevented[6].

The development of amrinone and its more potent analogue milrinone (both pyridinone derivatives) was followed by the synthesis and pharmacological characterization of many related positive inotropic/vasodilator agents: the imidazolones enoximone and piroximone, the pyridazinones pimobendan and imazodan, the quinazolinone quazinone, the pyrrolobenzimidazolone adibendan and others. It is thought that these drugs, by means of their common acidic amide function, compete with the phosphate function of cAMP for binding to the esteratic site of PDE III, thereby causing competitive inhibition of this enzyme and accumulation of cAMP[7].

Soluble and membrane-bound (sarcoplasmic reticulum?) cAMP-specific PDE III subtypes have been differentiated, the latter being the more important target for mediating a positive inotropic effect[8,9]. Figure 1 illustrates the subcellular site and mechanism of action of a prototype PDE III inhibitor, milrinone. Milrinone does not interact with Na, K-ATPase, the sodium channel, sodium–calcium exchange, receptors coupled to adenylate cyclase, or the calcium channel. Inhibition of the intracellular PDE III isozyme slows down the hydrolysis of cAMP.
and causes its accumulation until a new steady-state level is reached. cAMP activates protein kinases that phosphorylate the sarcolemmal calcium channel and phospholamban, a protein closely associated with the sarcoplasmic reticular calcium pump.

The positive inotropic effect

Cyclic AMP, by activating protein kinase, modulates sarcolemmal calcium channels (the L-type) such that more channels open during each action potential and the probability of opening of each individual channel during its burst-like activity is increased \(^{[10]}\). As a consequence, more calcium enters the cell during each cardiac cycle, and more calcium is released from the sarcoplasmic reticulum to activate the contractile proteins \(^{[11]}\). Intracellular injection of the bioluminescent protein aequorin allows the rise and fall of free intracellular calcium concentration (the calcium transient), which initiates and terminates myocardial contraction, to be recorded directly. All the positive inotropic PDE III inhibitors examined so far have been shown to increase the amplitude of the calcium transient, i.e. their positive inotropic effect can be clearly related to an increased intracellular calcium release as previously outlined (Table 1).

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
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<tbody>
<tr>
<td>Theophylline</td>
<td>Cat</td>
<td>12</td>
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<tr>
<td>Sulmazole</td>
<td>Dog</td>
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<td>Amrinone</td>
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<td>Milrinone</td>
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<td>Enoximone</td>
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<td>Piroximone</td>
<td>Ferret</td>
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<tr>
<td>OPC 8212</td>
<td>Dog</td>
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<td>Pimobendan</td>
<td>Ferret</td>
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A cAMP-mediated positive inotropic effect can be achieved by stimulators of adenylate cyclase such as noradrenaline, adrenaline, isoprenaline, dopamine or dobutamine, or by PDE inhibitors. While stimulation of adenylate cyclase by the catecholamine...
involves stimulation of beta-adrenoceptors, inhibition of PDE does not. Prolonged stimulation of beta-adrenoceptors induces their down-regulation and thereby terminates or diminishes the action of beta-adrenoceptor stimulants\textsuperscript{[19]}. This limitation is circumvented by PDE inhibitors, which bind directly to an intracellular enzyme rather than to extracellular beta-adrenoceptors.

In the intact cardiovascular system the inotropic activity of PDE III inhibitors is confounded by simultaneous decreases in preload and afterload (see below). If milrinone is infused into the left main coronary artery of patients such that approximate therapeutic plasma levels are achieved only in the coronary vessels and systemic vasodilation is therefore avoided, the drug does induce a positive inotropic effect as inferred from an increase in the maximum rate of left-ventricular pressure rise and stroke volume\textsuperscript{[20]}. These data indicate that a direct positive inotropic effect contributes significantly to the overall haemodynamic effect of milrinone in heart failure patients.

The positive lusitropic effect

Cyclic AMP-dependent protein kinases phosphorylate a key sarcoplasmic reticulum protein, phospholamban, and a subunit of the troponin-tropomyosin complex, troponin I, in addition to sarcolemmal calcium channels\textsuperscript{[21]}. These effects mediate a faster sequestration of released calcium through activation of the sarcoplasmic reticulum Ca-ATPase and a decrease in affinity of troponin C to calcium. Consequently, the rate of myocardial relaxation is enhanced and the duration of the relaxation process is shortened (‘positive lusitropic effect’)\textsuperscript{[22]}. This effect is physiologically important in that it preserves ventricular filling and coronary blood flow at the elevated heart rate induced by sympathetic stimulation. Additional, as yet unknown, modifications of the myofilaments may underlie the lusitropic cAMP effect\textsuperscript{[23]}. Evidence for a direct positive lusitropic effect of milrinone in heart failure patients has again been obtained by the intracoronary infusion technique: the positive inotropic effect was preceded by decreases in mean pulmonary capillary wedge and left-ventricular end-diastolic pressures\textsuperscript{[20]}.

Congestive heart failure is often associated with diastolic dysfunction\textsuperscript{[24]}. At the cellular level, the rate of decline in the intracellular calcium transient is drastically slowed in the ventricular myocardium of heart failure patients\textsuperscript{[25]}. The combination of positive inotropy and the shortening of relaxation time, which is confined to drugs acting via cAMP, may thus be expected to correct both systolic and diastolic dysfunction in heart failure patients.

Chrontropic, dromotropic and arrhythmogenic effects

The dependence on calcium channel activity of both the diastolic depolarization in the sino-atrial pacemaker cells and the conduction through atrioventricular nodal cells\textsuperscript{[26]} explains the positive chronotropic and dromotropic properties of cAMP-elevating drugs. Enhancement of atrioventricular conduction is observed with therapeutic doses of amrinone\textsuperscript{[27]} and milrinone\textsuperscript{[28]}. Significant increases in heart rate do not occur at therapeutic doses of amrinone or milrinone, but tachycardia does limit the dose range of PDE III inhibitors; 15 mg of orally administered milrinone induced tachycardia in some patients\textsuperscript{[29]}. Cyclic AMP increases cardiac automaticity and, at very high levels, may induce calcium overload with resultant triggered activity\textsuperscript{[30]}; both effects are arrhythmogenic. At therapeutic doses, however, the arrhythmogenic potential of milrinone is considered to be low\textsuperscript{[28,31]}.

Vasodilatation

Vascular smooth muscle contains a PDE III that is inhibited by cardiotonic PDE III inhibitors\textsuperscript{[32]}. As a consequence, the PDE III inhibitors induce accumulation of cAMP\textsuperscript{[33,34]} and relax arterial and venous smooth muscle. This relaxing effect is mediated by a decrease in the intracellular calcium level (amrinone\textsuperscript{[14]}, milrinone\textsuperscript{[35]}) which, in turn, presumably results from enhanced calcium extrusion across the sarcolemma. Vascular smooth muscle contains cAMP-dependent protein kinase which is known to stimulate a sarcolemmal calcium pump\textsuperscript{[36]}. In addition to raising cAMP, milrinone and related agents elevate the cGMP content in rat aorta\textsuperscript{[34]}, another second messenger known to relax vascular smooth muscle. As well as activating the sarcolemmal calcium pump, cAMP probably stimulates the sarcolemmal Na,K-ATPase. The resultant hyperpolarization and removal of intracellular sodium also serve to remove intracellular calcium and mediate relaxation. This could explain why ouabain, a cardiotonic steroid, diminishes the ability of milrinone and other cAMP-elevating drugs to relax vascular smooth
muscle. According to Harris et al., treatment of congestive heart failure with digitalis may actually attenuate the vasorelaxant potency of a number of therapeutically active vasodilators including PDE III inhibitors.

Cyclic AMP is the only known second messenger that combines positive inotropic and vasodilatory activity ('inodilation'). The underlying mechanism is a differential effect on cardiac and smooth muscle sarcolemmal calcium transport systems. Cyclic AMP leads to the activation of cardiac, but not of smooth muscle, calcium channels therefore promoting systolic calcium influx into the myocardiun. In smooth muscle, cAMP promotes calcium efflux across the sarcolemma. Other intracellular messengers, such as calcium itself or intracellular sodium ions (which are elevated by digitalis and activate a transmembrane sodium/calcium exchange in both heart and smooth muscle), combine positive inotropy with vasoconstriction. Unlike cAMP, the second messenger diacylglycerol activates calcium channels in both myocardial and vascular smooth muscle cells; this second messenger, therefore, also combines positive inotropic and vasoconstrictor responses. The combination of a positive inotropic effect with decreases in preload and afterload may explain why the increase in cardiac output induced by milrinone in heart failure patients is not associated with an increase in myocardial oxygen consumption.

Although few studies have addressed this point, experimental evidence indicates that the ratio of positive inotropic to vasorelaxant activity differs among the various PDE III inhibitors. In the isolated dog heart preparation coronary blood flow was increased to varying extents by inotropically equipotent doses of various PDE inhibitors, the order of effectiveness being amrinone > milrinone > sulmazole > piroximone > enoximone > OPC-8212. In the anaesthetized dog, milrinone and pimobendan were found to cause less peripheral vasodilatation than amrinone and enoximone at inotropically equipotent doses.

Cyclic AMP-mediated effects in other cell types

Platelet PDE III is potently inhibited by amrinone, milrinone, enoximone and related drugs. Antiaggregatory activity similarly has been demonstrated. In the case of amrinone, inhibition of thromboxane synthesis may also be involved. Possibly useful antithrombotic properties of PDE III inhibitors should therefore not be overlooked.

Leukocytes are another possible target of these agents. Therapeutic blood levels of amrinone inhibit the in vitro function (adherence and phagocytosis) of leukocytes. It is of interest in this regard, although speculative, that myocardial injury associated with reperfusion may result from an inflammatory process involving the migration and activation of neutrophils within the myocardiun. Simpson and Lucchesi have suggested that the reversible suppression of neutrophil activation during coronary thrombolysis or angioplasty is an important therapeutic objective.

The smooth muscle of airways is also relaxed by the cardiotonic PDE III inhibitors, although the affinity towards PDE III isolated from tracheal muscle has been found to be considerably lower than that towards the cardiac isozyme.

Relation to activity of adenylate cyclase

The synergistic, positive inotropic interaction between stimulators of adenylate cyclase, such as catecholamines and PDE inhibitors, has long been recognized and has been confirmed experimentally for many PDE III inhibitors. This synergism is due to the fact that the increase in the (steady-state) level of cAMP produced by a given reduction of hydrolysing PDE molecules is larger if the rate of cAMP production is increased (cf. Fig. 1). A synergistic inotropic interaction between dobutamine and milrinone is also apparent in the human heart, as demonstrated by the intracoronary application of these drugs. The addition of amrinone to intermediate doses of dobutamine during the intravenous treatment of severe heart failure produces a greater positive inotropic effect and improvement in left-ventricular performance than the administration of dobutamine alone.

A reduction in adenylate cyclase activity attenuates the degree of cAMP accumulation achievable with PDE III inhibitors. A pathologically reduced basal rate of cAMP production may underlie the in vitro finding that PDE III inhibitors have only a low inotropic efficacy on the excised ventricular myocardiun of heart failure patients. The clearly demonstrable positive inotropic effect of milrinone in patients (e.g. Ludmer et al.) may be explained by the in situ stimulation of adenylate cyclase, which results from the high circulating levels of catecholamines that characterize this condition.

Additional pharmacological properties

The therapeutic use of theophylline, a classical inhibitor of phosphodiesterases, is limited by serious
side-effects, tachycardia and arrhythmias[55] and a central stimulant effect that can lead to seizures and death[56]. This latter effect is unrelated to the inhibition of PDE but results from a blocking action on adenosine receptors in the CNS[57]. Notably, most of the newly developed PDE III inhibitors, including amrinone and milrinone[58,59], lack significant affinity to adenosine receptors and central stimulant effects. An exception is UD-CG 212 (an active metabolite of pimobendan) which has recently been shown to bind to rat adipocyte adenosine A1 receptors at micromolar concentrations[59].

Conclusions

Cardiac PDE III inhibitors derived from pyridinone, imidazolone, pyridazinone and related structures form a new class of positive inotropic/vasodilator agents that are useful in the treatment of acute and chronic heart failure. These drugs inhibit the intracellular hydrolysis of cAMP and thereby promote the cAMP-catalysed phosphorylation of sarcoplasmic calcium channels and activate the sarcoplasmic reticulum calcium pump in myocardial cells. The positive inotropic action is combined with positive lusitropic and vasodilator actions, the latter resulting from stimulation of calcium extrusion in vascular smooth muscle cells. The combination of positive inotropy with a reduction in preload and afterload may explain why these agents increase cardiac output in heart failure patients without increasing myocardial oxygen consumption. Milrinone and related drugs are devoid of the central stimulant action (adenosine-receptor antagonism) which narrows the therapeutic index of theophylline, central stimulant action (adenosine-receptor antagonism) which narrows the therapeutic index of theophylline. The effectiveness of these agents is, however, dependent to some degree upon adenylate cyclase activity. Thus, stimulation of adenylate cyclase (e.g. by dobutamine) simultaneously with the inhibition of PDE III (e.g. by milrinone) produces a synergistic positive inotropic effect.

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antagonist and functionally blocks the inhibitory

Discussion

PROFESSOR L. STORSTEIN (NORWAY)

As it has been shown that the half-life of milrinone
is very short, how often should it be administered?

PROFESSOR P. HONERJÄGER

Milrinone is usually given four times a day. I
understand that a slow-release preparation is being
developed.

UNIDENTIFIED SPEAKER

How has the vasodilatory effect of phosphodiester-
ase inhibitors been measured?

Has any distinction been made between direct and
indirect peripheral effects?

PROFESSOR P. HONERJÄGER

The vasodilatory effect has been established by
Japanese pharmacologists using isolated dog papil-
lary muscle and perfused isolated dog heart tissue,
indicating that the effect is a direct rather than an
indirect one. There have been few studies using an
intact organism, and it has not been established
whether different phosphodiesterase inhibitors have
different inotropic to vasodilatory activity ratios.