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Invited Paper

Is there evidence of a role of the phosphoinositol-cycle in the myocardium?

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Abstract

The recent findings on a more general involvement of phospholipids in signal transduction and on the different roles of inositolphospholipids in particular, thoroughly complicate research in this field. It becomes increasingly evident that measuring [^{3}H]inositolphosphate formation alone will never provide insight into the complex machinery of cellular signalling. Certainly for the heart in which the role(s) of the inositolphospholipids is far from clarified, the novel trends provide new directions for research.

Introduction

In recent years our knowledge of the signal transducing systems involving phospholipids has rapidly progressed. Especially the inositol containing phospholipids came into focus after the discovery of the second messengers diacylglycerol and inositoltrisphosphate [1, 2]. Since then, several hormones and neurotransmitters have been demonstrated to stimulate a phospholipase C in a receptor-mediated manner, apparently correlated with functional responses such as secretion, contraction and metabolic activities (for review see [3]). In the heart, similar correlates have been proposed, but their relevance remains far from clear. Recent findings on the involvement of different phospholipases in signal transduction and the putative role of the phosphoinositides in other cellular processes might add to the understanding of the complex machinery of transmembrane signalling.

In this paper I will discuss some representative data obtained from studies on the heart and highlight important new developments in phospholipid research.

Inositolphospholipids: chemistry and metabolism

Phosphoinositides constitute 2 to 8% of the cell membrane lipid. The most abundant form is phosphatidylinositol (PI) (Fig. 1), which in the sn-1 and sn-2 positions is esterified with two fatty acids. The sn-3 position is phosphodiesterified with myo-inositol at the D-1 position. PI can be phosphorylated to phosphatidylinositol-4 phosphate (PIP) by PI kinase [4, 5]. Very recently, it was found that a PI kinase (type I) could phosphorylate PI at the D3-position [6]. PIP kinase further phosphorylates PIP to phosphatidylinositol 4,5 biphosphate (PIP_2) [7]. These phosphorylation processes can be reversed by PIP_2- and PIP phosphomonoesterase [8] and appear on a metabolic scheme like futile cycles.

The regulation as well as the subcellular distribution of these phosphorylation-dephosphorylation processes is still unclear. Inositolphospholipids are substrates for different phospholipases [3, 9 10]. Especially PIP_2 specific-phospholipase C is under intensive investigation in view of its importance as a primary step after receptor activation. One of the phosphodiesteratic cleavage products is diacylgly-

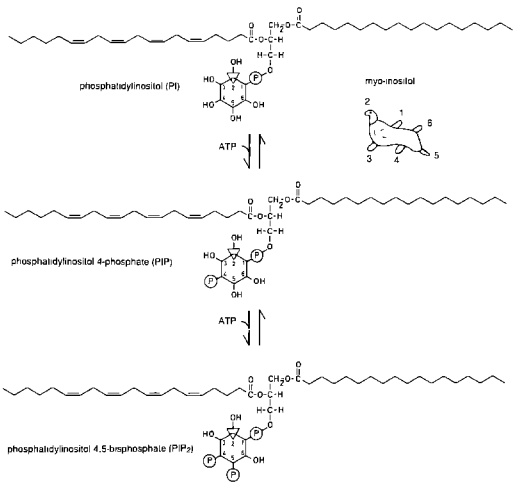

Fig. 1. The chemical structure of the inositolphospholipids. Myo-inositol is a hexahydroxycyclohexane. The molecule can be likened to a turtle in which the four legs and the tail are five equatorial hydroxyls, the turtle's head is the axial hydroxyl at position D-2 (▽) [87].

cerol, which activates protein kinase C, a key enzyme in this signal transducing system [1]. The remaining product, inositoltrisphosphate (IP_3) has been shown to mediate the release of Ca^{2+} from intracellular non-mitochondrial stores [2]. An IP_3-kinase has been identified in different tissues and phosphorylates this sugar phosphate at the D3-position; this tetrakisphosphate was reported to stimulate Ca^{2+}-influx through plasma membrane channels in oocytes [11]. These observations, which might be crucial in the understanding of how receptor activation leads the influx of Ca^{2+}, still needs confirmation in other cell types. The metabolic scheme for the resynthesis of inositolphospholipids is presented in Fig. 2.

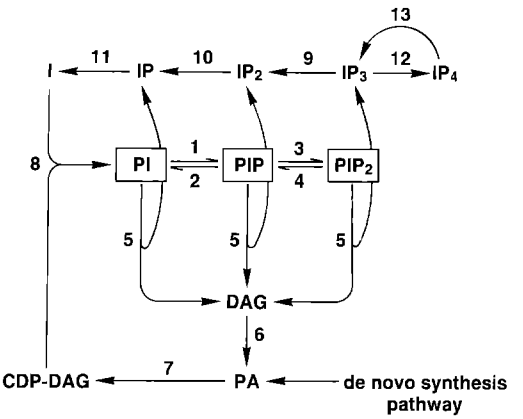

Fig. 2. Inositol phospholipid metabolism

PIP_2	: Phosphatidylinositol 4,5-bisphosphate
PIP	: Phosphatidylinositol 4-phosphate
PI	: Phosphatidylinositol
DAG	: Diacylglycerol
CDP-DAG	: Cytidine diphosphodiacylglycerol
PA	: Phosphatidic acid
IP_3	: Inositol trisphosphate
IP_2	: Inositol diphosphate
IP	: Inositol phosphate
I	: Inositol
1	: PI kinase
2	: PIP phosphomonoesterase
3	: PIP kinase
4	: PIP_2 phosphomonoesterase
5	: phospholipase C
6	: DAG kinase
7	: CTP-phosphate cytidyltransferase
8	: CDP:1,2-DAG inositol phosphatidyltransferase
9, 10, 11, 13	: Inositol phosphate phosphomonoesterases
12	: IP_3-kinase

Inositolphospholipids in the heart

Cardiac function is subject to control by the autonomic nervous system and the interaction between the sympathic and parasympathic nervous system is complex. In the heart, researchers have tried to correlate changes in mechanical activity with alterations in inositolphospholipid metabolism induced by different agonists. Interpretation of these data is hampered by the fact that the tissue preparations used for measuring mechanical activity, are very heterogeneous and contain, besides cardiac myocytes, also smooth muscle and endothelial cells, nervous and connective tissue, etc. The different cell types have their particular biochemical and functional responses when stimulated; likewise, the chemical changes measured in the total preparation do not necessarily correlate with the alteration in mechanical properties. Furthermore, species differences and the use of different cardiac tissue and cell preparations limits the number of general conclusions as yet to be drawn. I will therefore present some recent data that are representative for the work on the heart and should reflect its complexity.

α_1-Adrenergic response

By analogy with other tissues, α_1-adrenoceptor stimulation induces inositolphospholipid breakdown in tissue preparations [12–14] as well as in cardiac myocytes [14, 15]. In rat ventricular myocytes, epinephrine, the most potent agonist at eliciting [^{3}H]inositolphosphate release, is somewhat better than norepinephrine; phenylephrine and methoxamine are partial agonists [16]. The relative weak response of phenylephrine in inducing IP release contrasts with its efficiency in increasing the inotropy of the heart [17]. Recently Otani and coworkers [18] presented a study in which they compare the α_1-adrenoceptor mediated phosphoinositide breakdown with the inotropic response in rat left ventricular papillary muscles. In [^{3}H]inositol-labeled tissue, the authors found that phenylephrine induced a rapid decrease in PIP_2 and an increase in IP_3; PI was broken down slowly concomitantly with a long-lasting accumulation of IP. The mechanical response induced by the agonist consisted of a small transient increase in inotropy within the time scale of IP_3 formation, a subsequent drop in inotropic response followed by a long-lasting strong increase in contractile force. Phorbol-12,13-dibutyrate, a protein kinase C activator which, when given alone, had no effect on the inotropic response, strongly amplified the long-lasting inotropic effect of phenylephrine. As was suggested by the authors, these data are indicative of a role of Ca^{2+} mobilized by IP_3 in the fast, short increase in inotropy, while the sustained inotropic effect may be provoked by the activation of protein kinase C, consequent upon the long-lasting phospholipase C activity.

Muscarinic response

Stimulation of the cardiac muscarinic receptors also provokes the breakdown of inositolphospholipids, but in contrast to phenylephrine, induces a marked decrease in chronotropy and inotropy. At first glance it therefore appears that both neurotransmitters use the same transducing pathway to serve opposing physiological functions in the heart. However, the cardiac muscarinic receptors not only confine their primary message to inositolphospholipids, but are negatively coupled to adenylate cyclase by means of Gi, a guanine nucleotide binding protein [19]. Decreased formation of cAMP apparently diminishes the entry of Ca^{2+} through voltage-dependent channels [20, 21]. In the atrium, muscarinic receptors are also linked to K^+ channels, again by means of Gi and/or Go, without further involvement of a second messenger [22, 23]. Subsequent to receptor stimulation, K^+ conductance is increased, which leads to hyperpolarization of the sarcolemma and decrease in action potential duration [24]. Together with the reduced influx of Ca^{2+}, the increased influx of K^+ might contribute to a decrease in the mechanical activity of the heart. More insight in the relative importance of these primary biochemical alterations in cardiac function is obtained by comparing the potency of different muscarinic agonists in the separate responses and by studying their agonist concentration-dependency. Carbachol stimulation induces the hydrolyses of inositolphospholipid in a concentration range from 10^{-6} to 10^{-4} M [14, 25, 26]; decrease in Ca^{2+}-conductance [24] increase in K^+-conductance [24] and inhibition of isoproterenol induced cAMP formation [27, 28], are already obtained between 10^{-8} and 10^{-6} M of this agonist. Even more remarkable is the effect of oxotremorine, which effectively antagonizes contraction at the low dose range but is ineffective in provoking inositolphospholipid breakdown even at 3×10^{-4} M [25]. From these data it becomes evident that the negative inotropic and chronotropic effect of muscarinic agonists on the heart is probably not mediated by changes in inositolphospholipid breakdown. Evidence in favor of a causal relationship between inositolphospholipid breakdown and contraction, even when the muscarinic receptor was stimulated, was obtained in experiments in which the guanine nucleotide binding protein activation was blocked by pertussis toxin [25]. In these conditions, muscarinic inhibition of adenylate cyclase is abolished and the hyperpolarization resulting from increased K^+-conductance is inhibited. In chick atria, carbachol (10^{-6}–10^{-3} M) induces the hydrolysis of inositolphospholipid, depolarizes the

membrane and increases the contraction force [25]. Although acetylcholine itself is an agonist for the different biochemical and contractile responses described above, the functional significance of the positive inotropic effect of muscarine receptor stimulation in pertussis toxin-treated tissue is as yet not clear.

Other receptors coupled to inositolphospholipids

Besides the muscarinic and α_1-adrenergic receptor agonist, angiotensin [29], vasopressin [30], thrombin [31], adenine nucleotides [32] and atrial dilatation [33] have been reported to induce the hydrolysis of inositolphospholipid in cardiac cells. Although a stimulatory effect of this transducing system on the contractile response can not be excluded, it remains tempting to look for another common functional response. For the atrium, atrial natriuretic peptide (ANP) secretion might be such a response. Indeed, muscarinic agonists, adrenaline, vasopressin [34] and dilatation [33] have been demonstrated to induce ANP release. As found in many other cell types, inositolphospholipid breakdown might, at least in the atrium, be involved in stimulus-secretion coupling. Other roles for the PI pathway have been proposed, but for more information I would like to refer the reader to the excellent review by Brown and Jones [16].

From the experimental data presented in the literature, it is evident that cardiomyocytes carry receptors that induce changes in inositolphospholipid labeling. More insight into the molecular link between the phospholipid- and the functional response awaits further investigation.

Inositolphospholipids, a complicated matter

A major problem in studying the involvement of inositolphospholipids in signal transduction and its functional repercussion, is the often underestimated complexity of the inositolphospholipid metabolism itself. In the last part, I would like to focus on the new developments in lipid-mediated signal transduction and on the various 'non-second messenger' roles of the inositolphospholipids that might be implicated in cellular function.

Quantifying the formation of [^{3}H]inositolphosphates in [^{3}H]inositol-labeled tissue and cells is easy, inexpensive, and has definitely proven to be successful as a measure for phosphodiesteratic breakdown of inositolphospholipids. However, these measurements do not evidence the activation of a receptor-coupled inositolphospholipid-specific phospholipase C. This frequent overinterpretation becomes even more evident in the light of recent data on the existence of receptor-coupled (not inositolphospholipid-specific) phospholipase C [35–39]. The consequent abundant amount of DAG formed compared to the probably negligible amount of IP_3 likewise points to a major role of protein kinase C in triggering the functional response. Furthermore, receptor-mediated activation of inositolphospholipid kinases [40–44]is often overlooked. As long as the coupling (either mediated by a guanine nucleotide binding protein or not) of a receptor-type and the phospholipase C remains unproven, activation of inositolphospholipid kinases deserves consideration as primary steps. Indeed activation of these enzymes can lead to an increased formation of IP_3 and DAG due to the increased level of substrate for phospholipase C. These few examples of putative errors do not question the importance of IP_3 and DAG as second messengers, but emphasize the importance of careful biochemical analysis before the involvement of inositolphospholipid-specific phospholipase C is decided to be the primary step. Careful interpretation of the data that illustrate the activation of the second messenger 'acceptor' systems is important, too; that is to say the mobilization of intracellular Ca^{2+} and the activation of the protein kinase C. Although IP_3 has repeatedly been demonstrated to be selective among the inositolphosphates to induce Ca^{2+}-release from intracellular stores, evidence is accumulating that other metabolites such as GTP [45–47], NADP [48], phosphatidic acid [49] and arachidonic acid [50, 51] can mediate intracellular Ca^{2+} liberation. Especially the latter two might be important in signal transduction since their formation is also under receptor control. As for protein kinase C, the matter appears to be even

more complicated. Consistent with the recent identification of 7 subtypes of the enzyme that mainly differ in their regulatory region [52], evidence accumulates that regulating co-factors are also different. Again GTP [53] arachidonic acid [54–56], but also polyphosphoinositides [57], lipopolysaccharides [58], lysophospholipids [59], sphingomyelin [60] were shown to affect C kinase activity. Although the importance *in vivo* of most of these 'mediators' remains to proven, these data emphasize that activation of intracellular Ca^{2+} mobilization and of protein kinase C does not necessarily imply the activation of inositolphospholipid-specific phospholipase C.

Inositolphospholipids apparently have other functions besides being the substrate for second messengers. It is suggested that inositolphospholipids regulate protein kinases [61–63], DNA polymerase [64], hexokinase [65] and ATPase activity [66–69]. An outstanding example is the work of Vansanayi and his colleagues, which shows that skeletal muscle sarcoplasmic reticular Ca^{2+}-transport ATPase drastically increases when associated PI is phosphorylated to PIP [70]. Furthermore, polyphosphoinositides affect the assembly of actin [71–76]. The enzymatic activities mentioned above as well as the activation of the cellular contractile system are mostly under receptor control. Protein kinase C activity as well as changes in cAMP levels may affect the formation of polyphosphoinositides [77–81], which implies that receptor-mediated second messenger generation can regulate the level of inositolphospholipid and likewise enzymatic and/or contractile activity. A direct link between, however, remains to be proven.

Finally, it has been known for many years that glycosylated forms of PI serve as a covalent anchor for proteins (f.e. alkaline phosphatase, acetylcholinestase) to membranes (for review see [82]). More recently, glycosyl-PI was found to be involved in the action of insulin [83]. Both the protein-linked and the protein-free forms can be cleaved by phospholipase C, which yields diacyl-or dialkylglycerol. Free inositolglycan regulates at least in cell-free assays several insulin-sensitive enzymes such as cAMP phosphodiesterase, adenylate cyclase, pyruvate dehydrogenase and phospholipid methyltransferase [84–86]. A role for insulin-induced glycerolipid metabolism in mediating insulin-stimulated glucose transport in myocytes has recently been evidenced [87]. In future, inositolglycan as a novel second messenger will undoubtedly be the object of intensive research.

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