Conclusion

It can be stated that the signal-transducing system coupled to the serotonin-S_2 receptor involves inositol phospholipid breakdown. As far as we know, only for human platelets have the metabolic alterations in phospholipids, protein phosphorylation and Ca^{2+} metabolism been identified. The primary step (possible coupling by nucleotide-binding proteins and/or the first enzymatic step which becomes activated) remains to be elucidated. A more profound study of the metabolic steps involved in the phospholipid metabolism on activation of serotonin-S_2 receptors in other tissue is necessary to evaluate whether the same metabolic steps occur in the different tissue.

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Defective phosphoinositide metabolism in primary hypertension

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Summary. An increase in free cytosolic calcium content has been reported in essential hypertension. Since within the membrane, the phosphoinositides participate in the control of cell calcium homeostasis, we investigated whether impaired phosphoinositide metabolism could account for the calcium handling abnormality observed in hypertensives. In erythrocyte membranes of hypertensives the activity of kinases involved in polyphosphoinositide formation appears to be impaired and could be related to the alteration in calcium binding capacity and ATP-dependent calcium transport. In platelets of hypertensives, the hyperactivity of phospholipase C (observed even in the absence of calcium in the external medium) is likely to be responsible for the hypersensitivity of cells to various agonists. These observations are consistent with the hypothesis that in cells from hypertensives, a membrane defect linked to phosphoinositide metabolism is involved in the overall calcium handling defect.
Key words. Red blood cell; platelet; phospholipids; phospholipase C.

The increase in peripheral resistance that characterizes the established phase of primary hypertension results from an increased active tension in the vascular smooth muscle and this is likely to be a reflection of an increased concentration

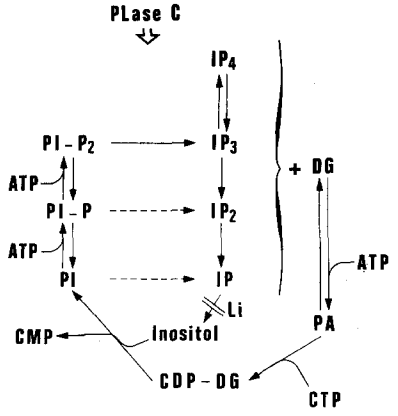
of free cytosolic calcium. Although it is not known how the intracellular calcium comes to be raised, abnormalities of calcium handling have been convincingly demonstrated in membrane preparations from various cell types in human essential hypertension and in spontaneously hypertensive rats (SHR)[6, 27]. Such membrane alterations seem likely to be of genetic origin, and it is tempting to postulate that they result from a same basic membrane defect. It has therefore been suggested that the sources of primary hypertension lie in a widespread alteration of the cell membrane function which affects the regulation of free cytosolic calcium concentration[30]. The phosphoinositides are described to be involved via their metabolism, in the control of transmembrane calcium fluxes and hence in the regulation of free cytosolic calcium concentration[3, 23, 34]. This prompted us to investigate whether the metabolism of phosphoinositides was altered in cells originating from hypertensive subjects (in comparison with normotensives) and whether this could be related to an impaired cell calcium homeostasis. Cellular calcium has been reported to be elevated in red blood cells, vascular smooth muscle cells and platelets of patients with essential hypertension and/or in SHR[5, 10, 19, 20, 32, 35]. Because blood cells are readily accessible, we studied the lipid metabolism in erythrocytes and platelets of SHR and of patients with essential hypertension.

The phosphoinositides and their metabolism

The phosphoinositides, i.e. phosphatidylinositol (PI) and its phosphorylated derivatives (phosphatidylinositol 4 phosphate (PI-P) and phosphatidylinositol 4,5-bisphosphate (PI-P2)) are ubiquitous glycerolipid components of membranes. Their polar head group consists of a myo-inositol ring (for PI) which can be esterified with one or two monoester phosphate groups to yield PI-P and PI-P2, respectively.

The phosphoinositide cycle is represented in the figure. The phosphoinositides are in equilibrium with each other owing to specific kinases and phosphomonoesterases which constantly add and remove phosphate groups from the 4- and 5-positions of the inositol ring of PI. The phosphodiesterase cleavage – by phospholipase C-like enzyme(s) – of phosphoinositides gives rise to the corresponding inositol phosphate and to 1,2-diacylglycerol (DG) which in turn is rapidly phosphorylated to yield phosphatidic acid (PA). As an intermediate in the phosphoinositide cycle, PA can be converted to cytidine diphosphate-diacylglycerol (CDP-DG) and then back to PI via the action of CTP : phosphatidate cytidyltransferase and CDP-DG : inositol phosphatidyltransferase, respectively.

The link between phosphoinositides and Ca^{2+} signaling has been recently elucidated when inositol trisphosphate (IP3), one of the products of PI-P2 hydrolysis by phospholipase C, was shown to function as a second messenger to mobilize Ca^{2+} [3]. In fact calcium-mobilizing agonists promote PI-P2 hydrolysis through the action of phospholipase C which cleaves the lipid to yield DG and the isomer IP3 (1,4,5), both of which appear to function as second messengers.



Schematic representation of major enzymic pathways involved in phosphoinositide metabolism.

As for other second messengers, IP3 (1,4,5) is produced rapidly and disappears equally as fast. The action of IP3 (1,4,5) is terminated by a specific phosphatase which removes the phosphate from the 5-position to give inactive inositol 1,4-bisphosphate (IP2). IP3 (1,4,5) can also be phosphorylated by a specific kinase to IP4 (1,3,4,5) which is the precursor of IP3 (1,3,4). IP2 is further degraded to yield IP1 and a lithium-inhibited inositol 1-phosphomonoesterase completes the sequence to yield free inositol which is reused for PI replenishment. When tested for their ability to mobilize calcium from intracellular stores, IP3 (1,4,5) was much more potent than IP3 (1,3,4) while IP4 (1,3,4,5) appeared to be ineffective[13]. Rather, IP4 (1,3,4,5) could act as a second messenger to control Ca^{2+} entry at the level of the plasma membrane[13].

One may envisage, therefore, that concomitant with Ca^{2+} mobilization, a change in plasma membrane Ca^{2+} transport occurs, which is associated with (and perhaps controlled by) phosphoinositide metabolism, as first suggested by Michell[23].

DG, the other product of the phosphodiesterase hydrolysis of PI-P2, is also rapidly degraded through the actions of DG lipase and DG kinase to yield monoglycerides and PA, respectively. As a second messenger, DG initiates the activation of a specialized protein kinase called protein kinase C. The signal pathway of this kinase is separate from and often synergistic with the Ca^{2+} signaling pathway of IP3. The second messenger function of DG through kinase C has been extensively and recently reviewed[25].

Relationship between phosphoinositide metabolism and other biochemical cellular changes or physiological responses

In healthy red blood cells, phospholipase C, which is directed against both PI-P2 and PI-P, does not function; the metabolism of phosphoinositides is therefore restricted to their interconversion owing to the rapid turnover of monoester phosphate group(s) or PI-P and PI-P2[1]. In erythrocytes, the major function of phosphoinositides is still unclear although a variety of roles have been suggested[1]. An increase in membrane content of polyphosphoinositides is apparently correlated with an increase in Ca^{2+}-ATPase activity and ATP-sensitive Ca^{2+} binding[15]. Moreover, the polyphosphoinositides appear to be capable of activating plasma membrane Ca^{2+}-ATPase by mimicking the effect of calmodulin[8].

In platelets, upon stimulation by a variety of agonists, the breakdown of PI-P2 a) appears to be the earliest event initiating cell activation[4], b) is responsible for the production of DG and IP3[21], and c) is associated with the release of dense granule constituents[16, 17, 27]. Evidence that the dense tubular system of human platelets responds to IP3 by releasing stored calcium was first presented by O'Rourke et al.[26]. Protein kinase C activation by DG induces the phosphorylation of a 40-K protein which is likely to be involved in granule labilization[11]. The time course of DG formation, 40 K phosphorylation and serotonin release are superimposable within the first 10–20 s following the activation of platelets with thrombin or collagen[31]. Ca^{2+} mobilization and C-kinase activation are synergistically involved in the physiological response of platelets. However, it has become clear now that protein kinase C can also provide negative feedback control over various steps of its own and other signaling

pathways, such as the receptors that are coupled to phosphoinositide hydrolysis [25].

In platelets, as in vascular smooth muscle cells, there are adenylate cyclase-coupled receptors. In both cells, an increase in cyclic AMP inhibits cell responses and modifies phosphoinositide metabolism [16, 17]. The interactions between the hormone-sensitive adenylate cyclase system and the phosphoinositide metabolism pathway have recently been reviewed [14].

The metabolism of phosphoinositides in blood cells of hypertensive subjects

Study of red blood cells

Lipid metabolism was investigated by measuring ^{32}P incorporation into inositol lipids following the incubation of isolated ghost membranes with (γ-^{32}P) ATP, and this was considered as an index of their turnover. More than 99% of the lipid-associated radioactivity was incorporated into PI-P2 and PI-P. ^{32}P-PI-P2 was decreased by 20–30% in adult and 3-week-old SHR compared to age-matched WKY; ^{32}P-PI-P was similar in both rat strains (table 1). Similar data were obtained with stroke-prone SHR (SHR-SP) [18]. Specific measurements of PI-P2-phosphomonoesterase and polyphosphoinositide phosphodiesterase activities did not show any difference between SHR and WKY [18]. Thus the altered phosphoinositide metabolism observed appears to be a consequence of some alteration in the activity of kinases which are involved in the phosphoinositide interconversion. Similar experiments performed on erythrocyte membranes isolated from patients with untreated essential hypertension also revealed an impaired lipid metabolism (table 1). Hypertensive patients whose blood pressure was lowered by beta-blocker therapy behaved as untreated hypertensives with respect to the ^{32}P-labeling of PI-P2 and PI-P [22]. Thus in hypertensives, whether treated or not, ^{32}P-PI-P2 was 28% higher than in normotensives (table 1). ^{32}P-PI-P also tended to increase in hypertensives although the difference was not statistically significant (table 1). Experiments performed in Sabra rats with DOCA/salt-induced hypertension gave results similar to those obtained in men [22]. As shown in table 1, ^{32}P-PI-P2 was lower in SHR whereas ^{32}P-PI-P2 was higher in hypertensive patients. We have no explanation for such a discrepancy, but because of the multifactorial nature of essential hypertension, it is conceivable that human essential hypertensives do not behave in the same manner as the various genetic animal models of hypertension.

Altogether, the results obtained from erythrocyte membranes indicate that the modification of phosphoinositide metabolism is not a consequence of blood pressure elevation, but can be rather considered as an intrinsic membrane defect. Keeping in mind that Ca^{2+} binding sites in inside-out erythrocyte membranes are 25–30% lower in SHR than in WKY [9] and that the increase in bound Ca^{2+} parallels the increase in ^{32}P-labeling of PI-P2 and PI-P [15], it seems likely that changes in phosphoinositide metabolism are associated with functional alterations of Ca^{2+} fluxes.

Study of intact platelets

The study of phosphoinositide metabolism, through ^{32}P-labeling of lipids, was performed after incubation of cells with ^{32}P-orthophosphate. The results in table 2 clearly indicate that the basal lipid turnover (i.e. that occurring in quiescent platelets) did not differ between hypertensive and normotensive subjects. This is in disagreement with those reported by Bühler et al. [6]. In contrast, upon thrombin stimulation (dose < 0.5 U/ml), the agonist-induced increase in ^{32}P-PA was markedly enhanced in SHR platelets (table 3). Under similar conditions, both aggregation and serotonin secretion were increased in SHR platelets (not shown). The fact that washed platelets from SHR were more reactive than WKY to a variety of agonists [2] suggests that the difference does not lie at the level of membrane receptors. Since ^{32}P-PA formation is known to be a sensitive index of phospholipase C activity [12], our results suggest that thrombin-stimulated phospholipase C activity is enhanced in SHR platelets. According to the mechanism of platelet activation (see above), this would result in an increased production of both DG and IP3; as a consequence, kinase C activity and Ca^{2+} mobilization would be enhanced. Consistent with this hypothesis, an enhanced kinase C activity has been reported in SHR platelets [33] and the thrombin-induced increase in free cytosolic Ca^{2+} was observed to be significantly higher in essential hypertensives [19].

Table 1. ^{32}P-labeling of polyphosphoinositides as a reflection of phosphoinositide metabolism in isolated red blood cell membranes

		^{32}P-PI-P2	^{32}P-PI-P
Rat			
3-week-old	WKY (8)	2.68 ± 0.23	1.83 ± 0.08
	SHR (8)	2.10 ± 0.17*	1.84 ± 0.08
12/15-week-old	WKY (6)	2.50 ± 0.20	0.43 ± 0.06
	SHR (6)	1.75 ± 0.14*	0.41 ± 0.05
Human			
Normotensives	(30)	0.92 ± 0.04	0.51 ± 0.03
Untreated hypertensives	(31)	1.18 ± 0.06**	0.63 ± 0.05

Results are expressed as ^{32}P nmol incorporated in 15 min/mg protein. Comparison with normotensive controls: * $p < 0.05$, ** $p < 0.001$, mean ± SE.

Table 2. Phosphoinositide metabolism in unstimulated quiescent platelets of WKY and SHR (A) and of patients with essential hypertension (B)

	^{32}P-PI-P2	^{32}P-PI-P	^{32}P-PI	^{32}P-PA
A)				
WKY (12)	34.9 ± 1.5	16.8 ± 0.6	26.6 ± 1.1	1.7 ± 0.1
SHR (12)	36.1 ± 1.0	16.3 ± 0.5	24.4 ± 0.9	1.8 ± 0.1
B)				
Normotensives (7)	39.1 ± 0.9	18.9 ± 0.5	31.2 ± 2.1	1.2 ± 0.1
Untreated hypertensives (5)	40.3 ± 1.0	19.7 ± 1.3	29.2 ± 3.1	1.6 ± 0.4

Results expressed as % of total ^{32}P-labeled lipids (mean ± SE).

Table 3. Thrombin-induced phospholipase C activity in SHR and WKY platelets as expressed by the thrombin-induced increase in PA labeling

Addition		^{32}P-PA WKY		^{32}P-PA SHR
no		100		100
Thrombin (U/ml)	0.1	152 ± 8 (8)	$p < 0.01$	198 ± 9 (8)
	0.2	221 ± 10 (8)	$p < 0.01$	307 ± 18 (9)
	0.3	282 ± 22 (9)	$p < 0.001$	444 ± 14 (8)
	0.5	459 ± 42 (7)	NS	520 ± 53 (6)
	1	727 ± 53 (3)	NS	630 ± 66 (3)

^{32}P-labeled washed platelet suspensions from WKY and SHR were stimulated with buffer (control) or thrombin (at the indicated dose) for 20 s. Results are expressed as % values of ^{32}P-labeled PA in stimulated samples versus the respective values in unstimulated controls, (mean ± SE).

Conclusion

The results that we obtained in erythrocyte membranes and in platelets cannot be compared since both the metabolic pathways and the role of lipid metabolism differ in these cells. Nevertheless it is obvious that the turnover of polyphosphoinositides is impaired in red blood cells from hypertensives. Phospholipase C is a key enzyme in the control of both cell Ca^{2+} concentration and cell reactivity to calcium-mobilizing agonists and its activity is not influenced by variations in cell Ca^{2+}. Accordingly, the difference (SHR vs WKY) in ^{32}P-PA that we observed after platelet stimulation by thrombin, did not change whether the external medium contained $CaCl_2$ or EGTA (not shown). In calcium-poor external medium, resting platelets from hypertensives and normotensives display similar free cytosolic Ca^{2+} level [20]. The impaired activity of phospholipase C appears therefore to be a primary membrane modification that could be responsible, at least in part, for the abnormal Ca^{2+} handling and hence for the hypersensitivity of platelets from hypertensives [2, 6]. Since platelets release a great number of vasoactive substances, the phospholipase C abnormality can be of relevance in the pathogenesis of hypertension.

Phosphoinositide hydrolysis has been shown to correlate with agonist-induced Ca^{2+} flux and contraction in rabbit aorta [7]. In addition, in smooth muscle cells from SHR both the resting cytoplasmic free Ca^{2+} level and the vasopressin-induced Ca^{2+} increase are enhanced [24, 32]. Nevertheless, one has yet to determine whether or not an impaired activity of phospholipase C could account for defective calcium handling in vascular smooth muscle cells of hypertensives.

The cellular mechanisms underlying the altered activity of platelet phospholipase C from SHR remain to be established. Recently the platelet cyclic AMP response to PGE1 was also demonstrated to be increased in essential hypertension [29]. One may therefore postulate that the abnormal enzyme activities are related to an inherent structural defect of the plasma membrane of hypertensives.

Abbreviations: PI, phosphatidylinositol; PI-P, phosphatidylinositol 4-phosphate; PI-P2, phosphatidylinositol 4,5-bisphosphate; PA, phosphatidic acid; DG, diacylglycerol.

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Platelet monoamine oxidase B: Use and misuse

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Summary. The human platelet in addition to having serotonin (5-HT) receptors, uptake carriers (receptor) and transmitter storage vesicles, primarily possesses mitochondrial monoamine oxidase (MAO) type B. Similar to the major form of MAO in the human brain, this enzyme actively oxidizes A-B and B substrates (tyramine, dopamine, phenylethylamine) as well as the novel secondary amine anticonvulsant, milacemide and dopaminergic neurotoxin, MPTP. 5-HT oxidation is hardly affected by the platelet enzyme and MAO inhibitors have no net effect on its accumulation. MAO-B is selectively inhibited by 1-deprenyl and thus the platelet enzyme may be useful to monitor the anti-Parkinson activity of such drugs, as related to their ability to inhibit brain MAO-B. The oxidation of the anticonvulsant, milacemide, to glycine in vitro and in vivo by MAO-B, may herald new prospects for the development of inert prodrugs capable of being metabolized to neuroactive substances by MAO-B. The plasma levels of their metabolites may be an index of MAO-B activity found in the platelet and brain.

Key words. Monoamine oxidase A and B; serotonin; phenylethylamine; milacemide; Parkinson's disease; epilepsy; anticonvulsant; platelet; clorgyline and deprenyl.

Introduction

The human platelet has proved to be an attractive, but limited, model of central serotonin (5-HT) neurones, because it possesses LSD and 5-HT receptors, 5-HT uptake transporter (imipramine binding sites)[21, 32, 34] and mitochondrial monoamine oxidase (MAO) B[19, 62, 65, 68]. However, unlike the 5-HT neurones it cannot synthesize the neurotransmitter since it lacks the rate-limiting enzyme, tryptophan hydroxylase[24]. Nevertheless, the platelet has been a useful organelle for the study of basic and clinical actions of psychotropic drugs[34, 44, 45]. The purpose of this paper is not to review the available literature on platelet monoamine oxidation in normal and pathological conditions, but to point out the use and misuse of it. For an extensive analysis of platelet MAO activity in psychiatric disorders, refer to a recent review by Fowler et al.[19].

Basic biochemistry of MAO

The outer mitochondrial membrane bound flavoprotein enzyme, MAO, catalyzes the oxidative deamination of a variety of primary amines including 5-HT, dopamine, noradrenaline, octopamine, tryptamine and phenylethylamine (table 1). This primary amine inactivating function of MAO gives the enzyme an important regulatory role within the aminergic neurones[30, 31, 62–64]. However, more recent observations clearly illustrated that the enzyme may have other functions unrelated to its ability of inactivating amine neurotransmitter substances in the CNS. Thus it can oxidize rather inert amines such as (a) MPTP (N-methyl-6-phenyl-1,2,3,6-tetrahydropyridine) to the selective dopaminergic Parkinson-inducing neurotoxin MPP^+ (N-methyl-6-phenylpridinium ion)[25, 36, 37] or (b) the novel anticonvulsant, milacemide (2-*n*-pentylaminoacetamide), to the inhibitory neurotransmitter, glycine[58, 72]. Therefore, MAO-B is endowed with properties which include amine detoxification as well as promotion of neurotoxin and neurotransmitter formation (table 1).

Table 1. Substrates and inhibitors of MAO-A and MAO-B

	MAO-A	MAO-B
Substrates		
Noradrenaline	+	
Adrenaline	+	
Serotonin	+	
Octopamine	+	
5-methoxytryptamine		+
Benzylamine		+
Phenylethylamine		+
p-methoxyphenylethylamine		+
MPTP		+
Milacemide		+
Dopamine	+	+
Tyramine	+	+
Inhibitors		
Clorgyline	+	
LY 51461	+	
Moclobamide	+	
Deprenyl		+
AGN 1135		+
MDL-72145		+
RO 16-6491		+
Milacemide		+
Tranylcypramine	+	+
Phenelzine	+	+

Taken from references 1, 25, 27, 37, 50, 55, 56, 58, 68, 70.

MAO exists as two catalytically active forms termed MAO-A and MAO-B[26]. The former is sensitive to inhibition by low concentration of the irreversible suicide acetylenic inhibitor clorgyline and the B form sensitive to inhibition by other acetylenic derivatives, 1-deprenyl[28] and AGN 1135[55, 64, 68]. The substrate and inhibitors of the two forms are presented in table 1. Distribution of MAO-A and MAO-B varies significantly in rat and human tis-