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COMMENTARY

PHOSPHOLIPID METABOLISM AND STIMULUS–RESPONSE COUPLING

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Excitable cells must have means for translating critical environmental changes into appropriate responses. One method for this involves the binding of extracellular stimuli to the cell surface and the subsequent issuing of intracellular signals that mobilize response mechanisms: surface membrane perturbation unleashes a pulse of informational molecules spreading throughout the cell. Many of these molecules are formed from resident, membranous phospholipids. These products do not exist preformed but rather are actively synthesized during cell stimulation. Platelet-activating factor (PAF) typifies this class of intracellular messengers. Platelets, leukocytes, macrophages, and endothelium produce PAF within seconds of stimulation. These same cells respond physiologically when challenged with the alkyl ether phosphatidylcholine [1], suggesting that PAF is an endogenous mediator that converts cell stimulation into cell function [2, 3]. However, more recent studies have argued against this hypothesis [4–9]. Such a polarization of views, which is common to the general field of phospholipid-derived mediators, reflects the complexity of stimulus–response coupling.

PHOSPHOLIPID-DERIVED MEDIATORS

Stimulated cells mobilize their phospholipids through at least three pathways to generate short-lived, bioactive intermediates [10]. In the first pathway, phosphatidylinositol (PI) is twice phosphorylated to yield PI-diphosphate; phospholipase C subsequently cleaves this product into diacylglycerol and inositol triphosphate (IP_3) (Fig. 1C) [11]. The

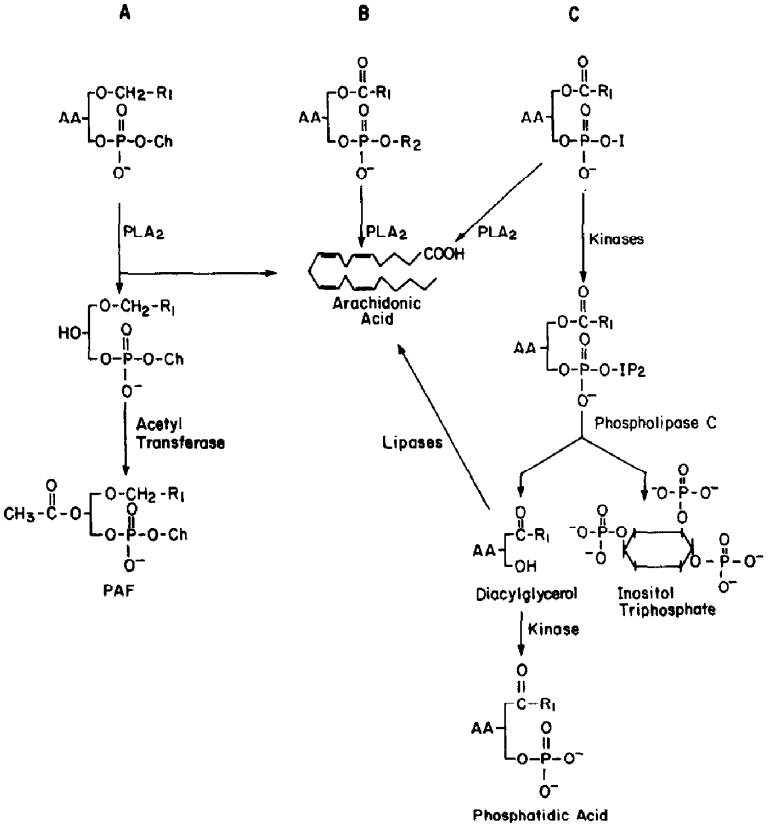

Fig. 1. Generalized scheme of phospholipid turnover in stimulated cells.

intramembranous diacylglycerol then binds with a soluble, cytosolic enzyme, protein kinase C (PKC), to form a membrane-associated macrocomplex that actively phosphorylates nearby proteins. Cytosolic Ca^{2+} promotes formation of this complex. Agents directly activating PKC are powerful, broadly bioactive agonists [12].

The other cleavage product of intramembranous PI-diphosphate, IP_3, diffuses to endoplasmic reticulum. There it binds with specific receptors and initiates the release of stored Ca^{2+} [11]. The subsequent elevation in cytosolic Ca^{2+} stimulates function, perhaps by influencing regulatory enzymes (e.g. PKC or phospholipases), contractile elements, or ion channels. IP_3 is also inactivated by cytosolic esterases, whereas diacyclglycerol is phosphorylated by a specific kinase. The respective products, inositol and phosphatidate, are combined enzymatically to regenerate PI and complete the "PI cycle". However, phosphatidate and its *sn*-2 deacylated derivative, lysophosphatidate, also activate cells. They may function directly or as components of specific ion channels to transport Ca^{2+} across membranous barriers [13, 14].

In the second metabolic pathway, phospholipases A_2 release arachidonate and other unsaturated fatty acids from phospholipids (Fig. 1B and 1A) [10]. These fatty acids are also hydrolyzed from PI by a specific phospholipase A_2, from phosphatidic acid by a second phospholipase A_2, or from acylglycerols by lipases (Fig. 1C). The release reactions are reversed by transacylases to complete a deacylation–reacylation cycle [10, 15–17]. Unsaturated free fatty acids stimulate cells, apparently by directly activating PKC [18–20]. However, one particular intermediate, arachidonate, is also metabolized to prostaglandins (PGs), thromboxanes (TXs), hydroxyeicosatetraenoates (HETEs), leukotrienes (LTs), and lipoxenes (LXs) (Fig. 2) [1, 21]. PGG_2, PGH_2, and TXA_2 activate various target cells by binding to specific receptors. These unstable products are rapidly converted to more long-lived compounds such as PGE_2, PGD_2, and TXB_2, which under certain conditions also appear capable of influencing cellular function [1].

LTB_4, a powerful stimulator of leukocytes and smooth muscle, operates through specific receptors which, at least in human neutrophils, are located in the surface membrane [22]. Some, but not all, responsive cells inactivate LTB_4 by oxidizing the molecule at carbon 20 (Fig. 2a). HETEs possess little or no agonist activity. 5-HETE, for instance, stimulates human neutrophils only at near micromolar and higher concentrations; it may indirectly activate PKC to initiate function.* 5-HETE, like LTB_4, is inactivated by terminal carbon oxidation; it is also rapidly acylated into cellular lipids.† LXs are newly described trihydroxy isomers formed by leukocytes stimulated under non-physiological conditions. Although not yet detected in physiologically challenged cells, these isomers have varying capacities to stimulate granulocytes, lymphocytes, and smooth muscle [21]. In a cell-free system, they activate PKC [20].

The third pathway produces PAF from resident alkyl ether phosphatidylcholine by the sequential actions of phospholipase A_2 and acetyltransferase (Fig. 1A). Acetylhydrolase and acyltransferases catalyze the reverse pathway and appear responsible for rapidly inactivating PAF [15–17]. The reversed limb of this cycle preferentially incorporates arachidonate [15, 17, 23, 24]. Furthermore, resting leukocytes and macrophages, but not platelets, contain appreciable quantities of 1-*O*-alkyl-2-arachidonoylglycerophosphocholine. There appears to be, then, an intimate association between PAF and arachidonate which, at least in certain cell types, ensures their concurrent production and inactivation. PAF stimulates a diversity of cells by binding to specific receptors. In the human neutrophil, these receptors appear associated with plasma membrane [25].

Many of the bioactive intermediates are themselves capable of stimulating phospholipid metabolism. PAF causes target cells to release fatty acids and metabolize endogenous arachidonate [16, 26]; it also triggers the PI cycle and thereby causes PKC activation [26–30]. PKC activators have similar effects on fatty acid release, arachidonate metabolism, and the PI cycle [31–33]. It is also important to note that a large number of intermediates mobilize Ca^{2+} by indirect, receptor-mediated (e.g. IP_3, LTB_4, and PAF [11, 26, 34]) or more direct (e.g. phosphatidate, lysophosphatidate, and oxygenated derivatives of unsaturated fatty acids [13, 14, 35]) mechanisms. Ca^{2+}-mobilization stimulates phospholipid metabolism as well as influences the actions of phospholipid-derived mediators. These many studies suggest that phospholipid metabolism is a potentially self-perpetuating, expanding series of metabolic steps that produces a large number and variety of bioactive intermediates. The situation is further complicated by the possibility that the intermediates cooperate in influencing function.

STIMULATORY INTERACTIONS

Calcium-mobilizing agents (e.g. IP_3) and PKC activators (e.g. diacylglycerols) stimulate prominent responses when used together under conditions in which neither type of compound has appreciable activity by itself [11, 12, 36]. Other combinations of the phospholipid-derived mediators demonstrate a similar type of synergy. For instance, the human neutrophil responds synergistically not only to direct Ca^{2+}-mobilizers and direct PKC activators [37–41] but also to the following combinations: PGE_2 and PKC activators; LTB_4 and PKC activators; 5-HETE and PKC activators; PAF and PKC activators; 5-HETE and LTB_4; and 5-HETE and PAF [41, 42,‡]. The 5-HETE–PAF interaction is illustrative. PAF degranulates cytochalasin B-pretreated neutrophils, whereas 5-HETE has little or no effect in this assay. Nevertheless, as little as 5 nM 5-HETE enhances degranulation responses to PAF; at higher concentrations, 5-HETE increases the potency of PAF

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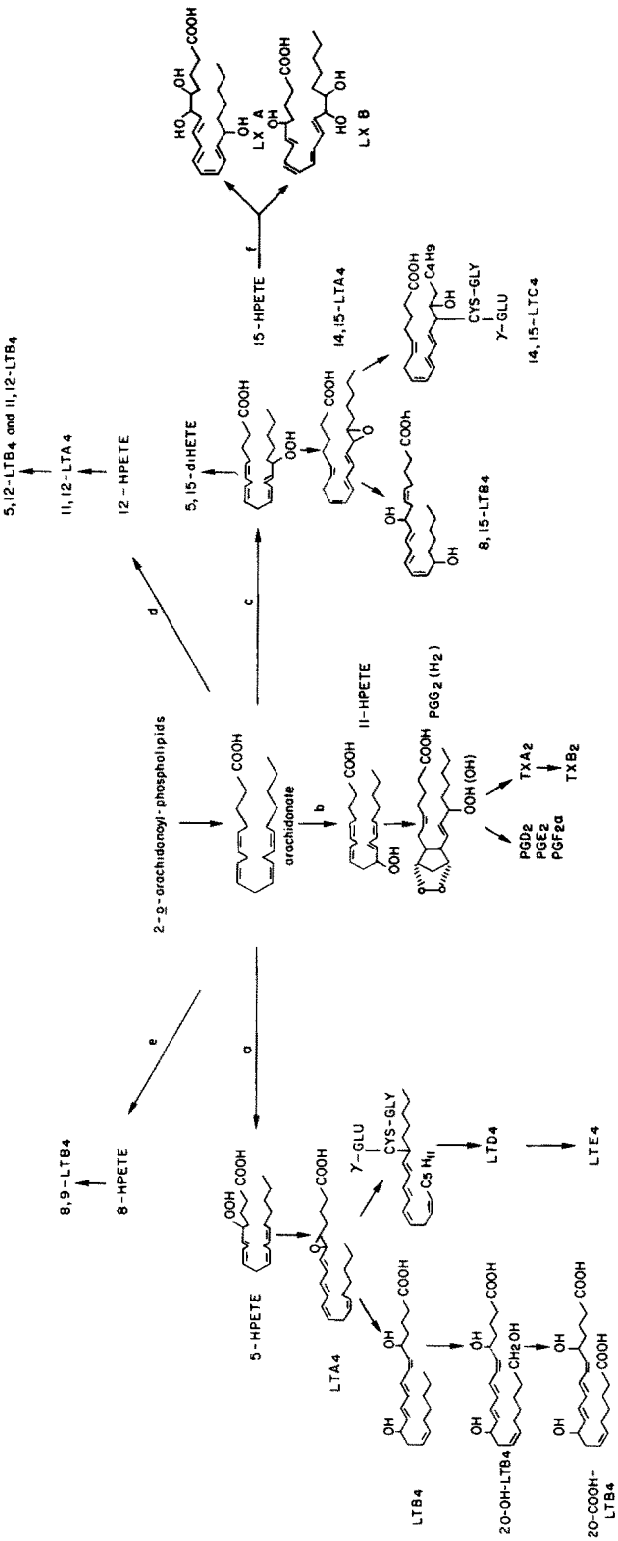

Fig. 2. Pathways of arachidonic acid metabolism.

almost 1000-fold [43]. The arachidonate metabolite can also enhance degranulation responses to other phospholipid-derived mediators such as LTB_4 and diacylglycerols but has no effect upon exogenous stimuli like chemotactic peptides [43, 44]. Furthermore, several other HETEs possess no synergistic activity and the PAF–5-HETE interaction is selective for degranulation responses: PAF-induced oxidative metabolism is less affected by 5-HETE (personal observations) and no synergy is evident in the neutrophil aggregation assay [44]. Finally, the combination of LTB_4, 5-HETE, and PAF produces a further increase in response magnitudes that is not explained by simple additive effects [45]. These findings prompt several general conclusions. First, seemingly inactive or weakly active mediators may possess unexpected potency when combined with other mediators. A phospholipid derivative cannot be discounted as a potential mediator simply because it lacks bioactivity by itself. Second, the actions and interactions of mediators may be assay-specific. Third, synergy can occur between three or more compounds; the number of possible combinations is huge. Lastly, the large number of interactions makes it difficult to determine the causes of any particular synergistic response. For instance, do synergistic interactions between direct PKC activators and direct Ca^{2+} mobilizing agents reflect the influences of Ca^{2+} on PKC activation or the stimulation of other events (e.g. production of LTB_4 or PAF) which may act in parallel with PKC to elicit function [46]?

INHIBITORY INTERACTIONS

Synergy follows simultaneous challenge with two or more stimuli. When the challenges are separated in time, an initially delivered stimulus may dampen subsequent responses. Thus, repetitive stimulation of a cell with the same or a structurally similar compound usually produces diminishing effects. However, this desensitized cell may be fully responsive to other agents. PAF, LTB_4, 5-HETE, PGs, IP_3, PKC activators, phosphatidates, and lysophosphatidates act in this way [1, 5–9, 47–56]. For instance, in assays of human neutrophil degranulation or oxidative metabolism, PAF desensitizes to itself, LTB_4 desensitizes to itself, but neither desensitizes to each other. Contrastingly, LTB_4 does cross-desensitize the cells to PAF in the human neutrophil aggregation assay [50, 52, 57]. Furthermore, the capacity of a non-physiological, Ca^{2+}-mobilizing agent, ionophore A23187, to induce human neutrophil degranulation is minimally influenced by desensitizing the cells to PAF, LTB_4 or 5-HETE. Nevertheless, the ionosphore loses >70% of its potency when neutrophils are desensitized to all three mediators [52]. It therefore appears that the same complex mediator interactions and assay specificities observed in stimulating cells can occur in inhibiting their responsiveness.

Desensitization has several explanations. It may reflect a functional inactivation of those specific receptors which bind the mediator [8, 58]. It may also result from inactivation of other receptors or the stimulation of events that counteract cellular responsiveness. For instance, PKC activators cause cells to become unresponsive to LTB_4 [59], PAF [60–62], and a large number of other stimuli [63–68]. In neutrophils, activated PKC enhances the function of surface membrane pumps to extrude Ca^{2+} [59, 64, 69]. The effect both blunts and rapidly reverses the rise in cytosolic Ca^{2+} induced by PAF [60–62] or other agonists [63–65, 67]. Furthermore, activated PKC phosphorylates and thereby appears to inactivate receptors for various exogenous stimuli [66–68]. In human neutrophils, PKC activation dramatically decreased the number of high affinity receptors for LTB_4.* Desensitization, then, may be due to: (a) a functional loss of receptors for the stimulating agent; (b) a loss of receptors for collateral mediators through which the test stimulus acts; and (c) a muted capacity of the desensitized cell to change cytosolic Ca^{2+}.

CONCLUSIONS

Cell types differ in the quantity and variety of mediators they produce. The idealized account given here neither reflects this nor includes all of the metabolic pathways, mediators, and mediator interactions that have been implicated in stimulus–response coupling. Even this limited overview, however, shows that three cyclic pathways can generate numerous intermediates with overlapping, complementary, and synergistic capacities to initiate or inhibit function. The signaling system appears redundant: each metabolic cycle produces intermediates that are fully capable of initiating function and also promoting further phospholipid mobilization. Coordinated responses to environmental stimuli may require such a complex, interwoven barrage of signals. In any event, we have not yet achieved an ordering of these signals into a precise, mechanical sequence that leads from cell stimulation to cell responses. Perhaps the system does not work in this way. Some responses may result from the turnover of PI, production of IP_3 and diacylglycerol, and the subsequent synergistic activation of PKC by IP_3-mobilized Ca^{2+} and diacylglycerol [10–12, 36–41]. Again turning to the human neutrophil, studies show that many physiological stimuli work without mobilizing [70] or requiring [71] the phosphorylating enzyme. Their actions, as well as those of agents which do appear to operate through PKC, were inhibited by arachidonate antimetabolites (the latter drugs inhibited the bioactions of indirect but not direct PKC activators.† Such results seem to indicate that (a) other mediators besides Ca^{2+} and diacylglycerol are involved in the physiological activation of PKC; (b) arachidonate metabolism may influence responses through PKC-dependent and -independent routes; and (c) cellular responses may be mediated by many different combinations of signals. Thus, in any given stimulus–response event, the plethora of potential mediators has multiple ways for initiating function; loss of any one mediator may be compensable by the actions of others. Selective inhibition of mediators either by pharmacological agents [18, 71, †] or desensitization techniques [4–9,

* J. T. O'Flaherty, J. F. Redman and D. P. Jacobson, manuscript submitted for publication.

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54, 58, 59] may produce little or no reductions in cellular responses. This lack of effect need not exclude a role for a putative mediator in the physiological response mechanism, given the enormous complexities of stimulus–response coupling. Obversely, the same techniques may lead to an inhibited cell [5, 7, 9, 50, 58–69, †] but, again, interpretation is clouded. A desensitizing stimulus may result in the functional loss of receptors for collateral mediators or the test stimulus; it may also reduce cellular responsiveness through more active mechanisms like promoting the extrusion of Ca^{2+}. Finally, pharmacological inhibitors will likely have unexpected effects upon such an intricate system. They may, for instance, block the inhibitory effects of mediators and thereby promote function. Desensitization remains a useful technique for demonstrating differences between stimuli, and pharmacological inhibitors have obvious clinical relevancies. Their use to dissect the events involved in stimulus–response coupling, however, offers numerous interpretive difficulties.

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