MINIREVIEW

Phospholipids and Electrolyte Transport (43150A)

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Over the last decade considerable advances in phospholipid biochemistry have shown that this class of lipids are not only important participants in the plasma membrane as a selective barrier, but are also involved in the transduction of receptor-mediated events and membrane permeability changes. In particular, the role of the acidic phospholipids, the phosphoinositides, and its second messenger generating system, inositol trisphosphate and diacylglycerol, have received substantial notoriety. This review will focus on the role of membrane phospholipids as it relates to ion transport processes.

Phospholipids as Membrane Barriers

Over the past 50 years, the importance of biomembranes in maintaining normal cellular function has evolved. The existence of bilayers in the plasma membrane and the presence of protein macromolecules representing channels and receptors has been firmly established using biochemical and physiochemical techniques (1). The concept of plasma membrane fluidity has originated from studies on the organization and phase characteristics of the membrane bilayer and from the segmental motion and from the transverse, rotational, and lateral mobilities of constituent lipids (2). Thus, the composition of the constituent lipids, phospholipids, and cholesterol serves to give some cells their unique characteristics and accounts for the differences in membrane capacitance and passive permeability properties as well as their responsiveness to external stimuli. It is well known that the membrane lipids not only represent a barrier for the entry and exit of molecules into cells but also provide a matrix for biochemical reactions to take place, to serve as recognition sites for receptors and, to contain domains for the endo- and exocytosis of macromolecules (3). Lipids in the plasma membrane are asymmetrically distributed (2), with almost all of the phospholipid molecules that contain a terminal primary amino group (phosphatidylethanolamine [PE] and phosphatidylserine [PS]) located in the inner half of the bilayer, while those lipids that contain choline (phosphatidylcholine [PC] and sphingomyelin) are found in the outer half of the lipid bilayer. Since the negatively charged PS is located in the inner monolayer, there is a significant charge difference between the two halves that may serve to enhance cation permeability. In addition, the calcium phospholipid-dependent protein kinase, protein kinase C, requires PS for activation, and since PS is in the inner membrane, it provides a means for the translocation of the enzyme from the cytosol to the membrane during activation. Thus, the asymmetrical distribution of the phospholipids is critical for signal transduction mechanisms. In addition to the asymmetry across the bilayer, there is heterogeneity among the different areas of the plasma membrane of the same cell (4). Thus, the distribution of any single phospholipid class may differ widely among different areas of the plasma membrane of a single-cell type although the total content of phospholipids may not change among similar cells. The renal brush border membrane, for instance, contains 1.64 times the cholesterol, 2.57 times the sphingomyelin, 1.98 times the PE, and 2.24 times the PS as the basolateral membrane, but only 0.42 times the PC and 0.41 times the phosphatidylinositol (PI) as the basolateral membrane (5). Thus, the ratio of individual phospholipid classes to the total phospholipid differs within membrane domains; such differences may impact on this areas permeability properties. In another renal model epithelia, the amphibian skin, no difference in the phospholipid fractions were noted between the ventral and dorsal skins of a variety of amphibian species, but the turnover of these phospholipids were signifi-

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cantly different and correlated to their permeability to water and responsiveness to vasotocin (6). In comparing the $^{32}P_i$ incorporation into phospholipids of the ventral and dorsal integuments of two species of frogs, our laboratory found that the ventral skin of *Hylidae cinerea* had a significantly greater incorporation of $^{32}P_i$ into the acidic phospholipids than its dorsal surface or that of the skin from *Rana pipiens* (Table I). The ventral skin of the Hylidae was sensitive to the actions of vasotocin, while the dorsal surface was not. In addition, the skin of the *R. pipiens* was also not as responsive as the Hylidae to the water flow response of vasotocin and there was no difference between the dorsal and ventral skins (Table I) (6). These observations suggested that the permeability properties of the integument paralleled changes in phospholipid turnover and that phospholipids may play a role in membrane permeability.

One of the basic functions of the plasma membrane is its barrier properties, where it limits the free diffusion of most solutes by a factor of 10^6–10^9. However, depending on the cell, certain solutes are selectively taken up into cells. This selectivity is due to the presence of specific "pores," carriers, and active transport processes in the membrane as well as endo- and exocytosis mechanisms that regulate large macromolecular transport into and out of cells. In epithelial cells, ion channels are present that also contribute to the selective nature of the plasma membrane and are often receptor regulated. Several studies have shown that phospholipids may play a significant role in the transport of ions and fluid across membranes (7, 8) and contribute to the cells permeability properties (9).

Phospholipids and Ion Translocation

The primary function of cellular membranes is the selective control of the movements of ions and other solutes and to serve as a messenger for the transfer of information from extracellular to intracellular compartments. Phospholipids have been suggested as being involved in the transport of ions across membrane lipid barriers (10) and contributing to the cells permeability properties (11). It has become increasingly clear that the intracellular concentration of calcium is dependent on the turnover of polyphosphoinositides in the plasma membrane, and that calcium mobilization is enhanced following the breakdown of phosphatidylinositol 4,5-bisphosphate (PIP_2) and the production of inositol 1,4,5-trisphosphate (IP_3) (12). In general, the concentration of calcium in the cell is several orders of magnitude lower than in the external media and a complex system of calcium transporters are present to maintain this calcium gradient. Early studies implicated phospholipid phosphatidate (PA) in regulating calcium fluxes across biologic membranes. Stimulation of a number of different cells by hormones or neurotransmitters yielded an increased intracellular calcium concentration that paralleled the formation of PA in the plasma membrane (13). In 1980, Salmon and Honeyman (14) demonstrated that addition of PA to isolated muscle cells rapidly produced cell contraction. The ability of PA to mimic endogenous stimuli was also found for a broad range of other cells by either addition of PA (15) or by generation of PA by addition of phospholipase (16). Tyson *et al.* (17), using a model of a biologic membrane, demonstrated that of all of the phospholipids tested only PA and cardiolipin were able

Table I. Changes in ^{32}P Incorporation into Phospholipids of Amphibian Epidermis as Compared with the Arginine Vasotocin (AVT) Hydroosmotic Response

Lipid fraction	Ventral skin		Dorsal skin	
	^{32}P Incorporation[a] (cpm/mg wet wt)	Water flow[b] (mg/cm²/hr)	^{32}P Incorporation[a] (cpm/mg wet wt)	Water flow[b] (mg/cm²/hr)
H. cinerea				
PIP_2	314 ± 2		61 ± 17	
PIP	360 ± 5	38 ± 6 (basal)	84 ± 30	9 ± 4 (basal)
PI	253 ± 14	222 ± 28 (+AVT)	84 ± 22	10 ± 4 (+AVT)
PA	138 ± 8		46 ± 13	
PC	221 ± 27		63 ± 20	
R. pipiens				
PIP_2	105 ± 17		145 ± 47	
PIP	83 ± 13	9 ± 0.09 (basal)	127 ± 51	8 ± 0.9 (basal)
PI	103 ± 9	19 ± 1.3 (+AVT)	144 ± 47	12 ± 2.0 (+AVT)
PA	101 ± 10		150 ± 46	
PC	84 ± 3		185 ± 56	

[a] Values are expressed as mean ± SE for six separate experiments and are given as cpm/mg surface skin wet weight. Tissue was incubated with 50 μCi of $^{32}P_i$ for 60 min at 25°C. Following this prelabeling period, the tissues were incubated for an additional 15 min before the reaction was terminated and phospholipids extracted (see ref. 6).
[b] Water flow was measured gravimetrically (see ref. 7) in the absence and presence of AVT.

to translocate calcium from one aqueous phase to another across the artificial lipid membrane. However, recent observations suggested that PA was an intermediate product of the agonist-induced cyclic breakdown and resynthesis of phosphorylated inositol phosphatides and that the product of PIP_2 breakdown, IP_3, mediates calcium mobilization (18). Moolenaar *et al.* (19) have provided some additional controversy as they confirmed that PA addition to cultured cells as well as phospholipase D treatment resulted in an increase in the intracellular concentration of calcium. Moreover, phospholipase D activity has been identified in mammalian cells and may have as yet an unknown role in calcium homeostasis. It could be that PA is converted to diacylglycerol by a phosphatase, resulting in the activation of protein kinase C. Activation of this kinase may modify calcium channels and calcium transporters in regulating intracellular calcium concentrations. As calcium ions have a high affinity for acidic phospholipids present in the membrane interface, it has been suggested that calcium may be translocated preferentially across the plasma membrane through this lipid interaction (20).

However, calcium is not the only ion that has been linked to changes in phospholipids; in the 1970s our laboratory demonstrated that phospholipase C stimulated sodium transport across the amphibian integument (21) and the crystalline lens of the toad (22). The increase in sodium transport observed with phospholipase C on frog skin was associated with an increase in the influx of sodium with no change in efflux (21). This response was inhibited by amiloride, a diuretic which specifically blocks sodium transport in epithelial membranes (23). It was suggested that phospholipase C may function in controlling membrane permeability and provide a "model" for hormonal effects. Subsequently, our laboratory demonstrated the presence of phospholipases in another transporting epithelia, the toad urinary bladder (24). In addition, our laboratory has shown that the phospholipid metabolite, diacylglycerol, stimulated transepithelial sodium transport across the isolated amphibian skin (25) and U18666A, an inhibitor of sterol biosynthesis and phospholipid turnover (26), inhibited sodium transport across the skin of the frog, *R. pipiens* (6). These studies suggested that phospholipids may play an important role in the transport of sodium in epithelial membranes. Consistent with these findings was the observation that a significant correlation existed between sodium conductance and phosphatidylcholine content in the epidermis of the frog, *R. pipiens* (27). These authors further demonstrated that following high salinity adaptation the lipid transport correlation was absent and sodium conductance was suppressed (28). In addition, it was reported that there was a relative increase in membrane sphingomylin concentrations following high salinity adaptation. This was interesting, as more recently sphingomylin has been shown to produce a marked inhibition of intracellular phospholipases and that this lipid may play a role in stabilizing the phospholipid structure in the bilayer (29). Thus, a positive correlation exists between changes in membrane phospholipids and the state of transepithelial sodium transport in amphibian epithelia.

Hormones, such as antidiuretic hormone (vasopressin) and aldosterone, also enhance sodium transport across amphibian epithelia as well as renal tissues. Previously, our laboratory has reported that mepacrine, a phospholipase inhibitor, attenuated the increase in sodium transport mediated by aldosterone in toad urinary bladder preparations (24). The current scheme for the increase in sodium transport produced by aldosterone on renal epithelia is shown in Figure 1. The predominant action of aldosterone is an increase in the apical membrane sodium conductance that occurs through amiloride-sensitive channels. A number of different glycoproteins are induced by aldosterone, includ-

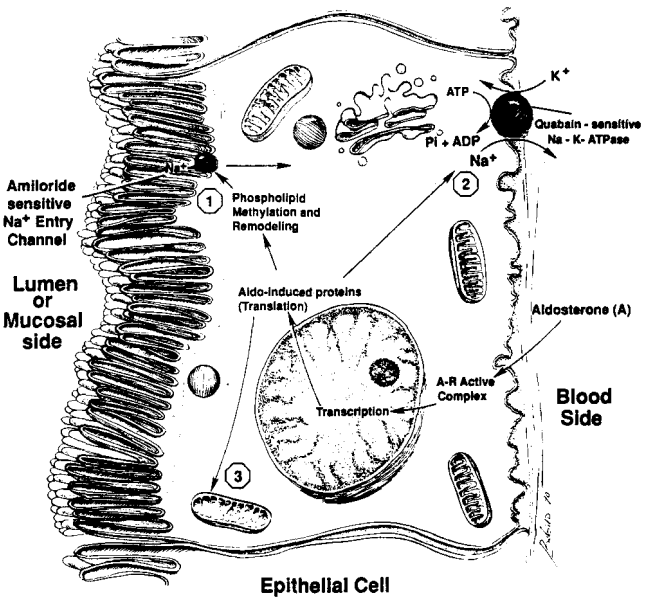

Figure 1. A diagram of the proposed sites and mechanisms of the mineralocorticoid hormone, aldosterone, actions. The hormone binds to its cytoplasmic receptor, and the activated receptor complex diffuses into the nucleus where it binds with high affinity to chromatin and activates the gene expression of aldosterone-induced proteins. These aldosterone-induced proteins ultimately bring about the physiologic response of enhanced sodium reabsorption. Site 1 represents the activation of amiloride-sensitive channels in the apical membrane that are present in an inactive state. This site includes a methylation step and membrane lipid remodeling that is associated with enhanced sodium conductance of the apical membrane. Site 2 represents aldosterone effects on the Na^+-K^+-ATPase that may involve an increase in the synthesis independent of any effects on apical sodium permeability. Site 3 indicates the aldosterone response is dependent on metabolic energy and involves the induction of mitochondrial enzymes (100).

ing GP70, a protein that can be localized to the apical plasma membrane of toad urinary bladders (30) and may be a component of the apical amiloride-sensitive channel (31). Aldosterone increases sodium conductance by activating preexisting sodium channels (32, 33), possibly involving methylation of a membrane component, including phospholipids (34). The involvement of lipid metabolism in the actions of aldosterone was first put forth by Goodman *et al.* (35), who demonstrated that aldosterone produced significant effects on fatty acid biosynthesis and phospholipid remodeling. In addition, tetrahydrol-naphthyl, phenoxy proprionic acid (TPIA), an inhibitor of the rate-limiting enzyme in fatty acid synthesis (acetyl-CoA carboxylase), prevented the rise in sodium transport following aldosterone (36). Subsequently, Weismann *et al.* (37, 38) reported that phospholipid methylation was involved in the increase in sodium transport by aldosterone. This same laboratory demonstrated that 3-deazoadenosine, an inhibitor of phospholipid methylation, attenuated aldosterone increase in sodium conductance (34). Such actions of aldosterone on membrane phospholipids have been suggested by Marver (39) to represent a membrane remodeling cycle as part of the sequence of events initiated by aldosterone. Additional observations on renal inner medullary cells support the concept of membrane remodeling, as phospholipid methylation enhanced arachidonic acid release and prostaglandin synthesis through activation of phospholipases (40). Collectively, these observations indicated a role for phospholipids in the enhanced sodium reabsorption by aldosterone, and that phospholipids and phospholipid turnover may be involved in regulating or maintaining sodium transport in these epithelia.

Membrane Lipid Composition and Changes in Ion Transport

As suggested above, evidence has accumulated over the last 20 years that has dispelled the long held dogma that phospholipids in cell membranes are primarily relegated to the rigid bilayer structure which acts as a permeability barrier against ion and molecular transport. Many recent studies have revealed that changes in membrane composition of phospholipids may be induced by various hormonal or metabolic changes and this can lead to alterations in ion transport. Tyson *et al.* (17) demonstrated that transport of Ca^{2+} across an organic phase separated by two aqueous phases could be stimulated by the addition of the phospholipids, cardiolipin and PA. These two phospholipids were found to be equal in ionophoric activity to X537A, a potent calcium ionophore. In a later study Green *et al.* (41) also demonstrated that several different phospholipids increased the transport of Ca^{2+}. Among the most active phospholipids in this regard were PA and PI. Additionally, in this same study, these authors were able to demonstrate that lysolecithin was an effective ionophore for a plethora of compounds including ions, amino acids, histamine, and organic acids. These studies provided the initial hypothesis that phospholipids or their metabolites may function to alter ion translocation.

Subsequently, many hormones and neurotransmitters were identified that appeared to act through alterations in lipid turnover and composition. It has been shown by Klahr *et al.* (42) that parathyroid hormone (PTH) will increase the turnover rate of membrane phospholipids. Studies by Islam *et al.* (43) have further revealed that during chronic renal failure, which is manifested by high concentrations of PTH, there was a significant reduction in total phospholipids, PI, PS, and PE, in brain synaptosomes. Additionally, they have demonstrated that this change in phospholipid content, brought about by increased PTH concentrations, leads to Ca^{2+} accumulation in brain synaptosomes and eventually leads to abnormalities in the neurotransmitter function of these units during chronic renal failure. In a later study, this same group demonstrated that the abnormalities in calcium and phospholipid content of the synaptosomes could be reversed by treatment with verapamil, a Ca^{2+} channel blocker (44). Similarly, it has been reported (45) that PTH as well as Ca^{2+} itself stimulated ^{32}P incorporation into red blood cell membranes. Specifically this study demonstrated that PTH stimulated an increase ^{32}P incorporation into PI. This, in turn, could lead to increased Ca^{2+} flux into the cell and increase the rigidity of the red blood cell membrane.

Phospholipids also appear to play an important role in the metabolism of the kidney. The inositol phospholipids, in particular, have been implicated through a growing body of evidence that indicated increased phosphoinositide metabolism in the kidney was linked to cellular calcium mobilization and to the activation of protein kinase C (46). Several reports have demonstrated that PTH stimulated IP_3 and diacylglycerol formation in renal tubular cells (47, 48). In a recent review, Menê *et al.* (10) point out the importance of the hydrolysis of the membrane phospholipids, PI, PC, and PE, as playing a central regulatory role in normal functions of the mesangial cell. A study by Wirthensohn *et al.* (50) examined the effects of several hormones on phospholipid metabolism in rat kidney tubules. They found an increased ^{32}P incorporation into PC and PI when the tubules were stimulated with angiotensin II, phenylephrine, or PTH. Their conclusion was that phospholipid turnover and metabolism was a very active metabolic process in renal tubules and was under specific hormonal control.

Yorio *et al.* (51) have shown in the toad urinary bladder, an analog to the mammalian distal nephron, that antidiuretic hormone stimulated permeability

changes may be mediated by a breakdown of PA and PI. This breakdown ultimately leads to activation of protein kinase C, which modulates the hormone-mediated response. In addition, Yorio *et al.* (6) have demonstrated, in the amphibian skin, that alterations in phospholipid turnover and/or content can modify epithelial membrane permeability to ions and fluid and may play an important role in the regulation of hormone-mediated permeability changes.

The transport of sodium by epithelial cells is known to be modified by changes in turnover or composition of membrane phospholipids. Goodman *et al.* (35) were first to report that aldosterone could induce changes in lipid metabolism in the toad urinary bladder. They were able to show that aldosterone increased conversion of [^{14}C]pyruvate into fatty acids, that there was newly synthesized lipid in the 2-position of phospholipids, and that there was a compositional change in the fatty acid in the phospholipid fraction of the tissue lipids. All of these findings were associated with the aldosterone-stimulated increase in Na^+ transport. In two later studies (36, 52) this same laboratory confirmed these earlier findings and in addition was able to demonstrate by using an inhibitor of acetyl-CoA carboxylase, TPIA, and a sodium channel inhibitor, amiloride, that the change in membrane lipid metabolism seen with aldosterone was a primary response to the hormone and not secondary to stimulation of sodium transport. Additional studies with the A6 amphibian kidney cell line (34) suggested that the methylation of apical membrane phospholipids was also involved in the increase in apical sodium transport that aldosterone induced. Other reports (39) confirmed this finding and further showed that a methylation inhibitor, 3-deazaadenosine, inhibits methylation of phospholipids as well as the aldosterone-dependent rise in short-circuit current.

Changes in phospholipid metabolism have also been implicated in several H^+ secreting tissues, namely, gastric mucosa and renal epithelia. It has been known for sometime that the gastric mucosa contains a H^+-K^+-ATPase (53) and that the activity of the ATPase may be related to its lipid environment. A report by Olaisson *et al.* (54), using gastric vesicle membranes, demonstrated that phospholipase C degraded 50% of the membrane phospholipids, leaving only sphingomyelin and PI intact. Concomitant with this breakdown was a 50% reduction in the H^+-K^+ ATPase activity.

An early report in the literature (55) indicated that with a change in metabolic state (i.e., metabolic acidosis) which required an increased secretion of H^+, there was a concomitant increase in the total lipid of rat kidneys. Following on this lead, Frazier and Yorio (56) were able to demonstrate in urinary bladders from toads in metabolic acidosis that there was an increase in certain fractions or percentage of composition of membrane phospholipids (Table II). Additionally, Frazier (57) was able to demonstrate that insulin and deoxycorticosterone were both capable of inducing changes in membrane phospholipids of toad urinary bladder. Both insulin and deoxycorticosterone are known stimulators of H^+ secretion in this epithelia (58, 59). This change in phospholipid turnover or percentage of composition in toad urinary bladder as reported above in Table II appeared to parallel changes in proton secretion; however, the mechanism by which this change in phospholipid metabolism stimulates H^+ secretion remains uncertain. The changes in phospholipid turnover could be stimulating the proton-ATPase directly or perhaps could be involved in membrane remodeling where new phospholipid could be incorporated into the membrane and the altered lipid environment then augments the proton transport.

Table II. Changes in Membrane Phospholipid Turnover or Composition by Various Metabolic or Hormonal Pertubations in Toad Urinary Bladder

Treatment	Phospholipid fractions					
	PE	PC	PA and PS	PI	PIP	PIP_2
Metabolic acidosis						
Turnover	↑[a]	NC[b]	↑	NC	NC	NC
%	↑[c]	NC	NC	↑	NC	NC
Insulin (100 milliunits/ml)						
Turnover	NC	↑	NC	↑	NC	NC
%	NC	NC	NC	NC	NC	NC
DOCA (10^{-6} *M*)						
Turnover	NC	NC	NC	NC	NC	NC
%	NC	↑	NC	NC	NC	NC

[a] Increased turnover was measured from incorporation of ^{32}P.
[b] NC, no change (taken from refs. 56 and 57).
[c] Increased percent was measured as percentage of total lipid phosphate.

Phospholipids as a Source of Second Messengers in Ion Transport Processes

Since the initial observations of Hokin and Hokin (60) that acetylcholine stimulated the incorporation of ^{32}Pi into PI and PA in pancreatic slices, a variety of receptors have been identified as being linked to the metabolism of polyphosphoinositides and the generation of a calcium signal (12, 61). Thus, it has been established that membrane phospholipids serve as a source for receptor signaling in cells and that the products of phospholipid metabolism have second messenger functions. This response, which was known as the "phospholipid effect" has subsequently been demonstrated for a variety of tissues and receptor subtypes, including muscarinic-cholinergic, α_1-adrenergic, dopaminergic, vasopressin (V_1), and other active peptides (12). For details concerning these receptor mechanisms please consult recent reviews (12, 61, 62). The second messengers derived from phospholipid hydrolysis in-

crease intracellular calcium concentrations and activate a phospholipid-dependent protein kinase, protein kinase C (PKC). The increase in calcium results from the actions of IP_3 and the activation of PKC through the release of diacylglycerol, both products of phospholipase C action on phosphatidylinositol 4,5-bisphosphate. Of particular importance to the regulation of ion transport has been the observations that activation of protein kinase C regulates sodium (63, 64), potassium (63), and chloride transport (64, 65). With the use of *in vitro* microperfusion techniques, Hays *et al.* (63) found that activation of PKC with either phorbol 12-myristate 13-acetate, or L-α-1,2-dioctanoylglycerol significantly inhibited net sodium absorption, net potassium secretion, and transepithelial voltage in a dose-dependent manner. Similarly, inhibition of sodium transport by PKC activation has been observed in A6 kidney cells in culture (64) and was attributed to a decreased sodium conductance of the apical plasma membrane. In rabbit proximal colon, PKC activation reportedly inhibited sodium-hydrogen exchange and decreased intracellular pH as measured by 5(6)-carboxyfluorescein diacetate, an intracellular fluorescent pH probe (66). The mechanism whereby PKC may regulate ion transport is not completely understood; however it could act directly on the ion channels by phosphorylating specific protein constituents, as has been suggested for PKC inhibition of Na^+-K^+-$2Cl^-$ cotransport in BALB/c3T3 cells (67). In addition, activation of PKC appears to be involved in cellular acid base regulation, particularly with respect to acidification processes in epithelial cells. In turtle urinary bladder, activation of PKC inhibits H^+ secretion by the apical proton pump (68) and recently our laboratory has reported similar inhibitory effects of PKC activation in another renal type epithelium, the frog skin (69). In frog skin, apical proton excretion was inhibited by phorbol 12-myristate 13-acetate and mezerein, a nonphorbol activator of PKC. These structurally different compounds produced similar effects whereas the inactive epimer of phorbol 12-myristate 13-acetate was without an effect. In toad urinary bladder, however, enhanced acidification was seen with the nonphorbol PKC activator mezerein (70). A summary of the effects of activating PKC on ion transport processes is presented in Table III. It appears that phosphorylation of ion channels by PKC may be one means whereby cellular self-regulation of ion transport occurs in renal type epithelia. As several hormones which alter renal electrolyte transport appear to act through their respective receptors coupled to phospholipid hydrolysis (vasopressin, bradykinin, parathyroid hormone, angiotensin II), activation of PKC may be an integral part of the hormonal control of electrolyte transport, intracellular pH, and volume regulation in renal type epithelia. The hydrolysis of membrane phospholipids and activation of lipid second messengers thus plays an essential role in the ion transport changes induced by these hormones.

The most widely studied ion transport systems affected by phospholipid second messengers is calcium transport and calcium mobilization. Michell (13) put forth in the 1970s the hypothesis that hormones and neurotransmitters increase intracellular calcium through phospholipid signaling. Such an hypothesis has received considerable support over the years. The finding by Berridge (18) that IP_3 mobilized intracellular calcium has provided the link between hormones and calcium signaling that was missing. The increase in intracellular calcium that occurs following agonist stimulation has also been shown to be involved in the regulation of other ion transport processes in the cell either through direct effects or indirectly by enzymes involved in phosphorylation (71). The role of calcium in the control of cellular ion transport is beyond the scope of this review; however, the rise in intracellular calcium does have additional effects on phospholipid metabolism through the activation of lipases. In particular, it has been shown that following a rise in intracellular calcium there is a concomitant increase in free arachidonate, the precursor for prostaglandins and leukotrienes. The free arachidonate is derived from either the actions of phospholipase A_2 on membrane phospholipids, or derived from 1,2-diacylglycerol (72), a product of phospholipase C activity. In some tissues the release of free arachidonate occurs from the sequential release of the 1-steroyl and then the 2-arachidonyl residue (73). In addition, arachidonate can be derived from PC as a result of the activation of phospholipase A_2 (74). Prostaglandins that are released as a result of these lipases have dramatic effects on ion transport processes in epithelial membranes.

Prostaglandins (PG) are ubiquitous substances, being present in most all body tissues. All PG have as a common precursor arachidonic acid, which is found in abundance at the 2-position of many membrane phospholipids. Following appropriate stimulation, the arachidonic acid can be liberated from the phospholipid as a free fatty acid by action of the activated enzyme phospholipase A_2 or as stated above from 1,2-diacylglycerol. This arachidonic acid released from the cell membrane is the rate-limiting step in the formation and release of PG from the cell. Most cells contain adequate amounts of cyclooxygenase enzyme which quickly converts the arachidonic acid into the PG cascade. PG function in a classical sense as autocrine or paracrine hormones and have a multitude of physiologic effects within an organism.

Phospholipase A_2 activity and the formation of PG through the cyclooxygenase system have been shown to be present in mammalian kidney (75, 76). Likewise, toad urinary bladder and frog skin, analogous epithelial tissues to the distal nephron, have been shown to have

Table III. Relationship between PKC Activation and Ion Transport

Ion	Tissue	PKC Activation (phorbol esters or mezerein)	Reference
Na^+	Cortical collecting tubule	↓[a]	(63)
	A6 kidney cells	↓	(64)
	LLC-PK, porcine kidney cells	↓	(96)
	Frog skin	↑[b]	(97)
Na^+-H^+ exchange	Proximal colon	↓	(66)
Na-K-2Cl	BALB/c3T3 cells	↓	(67)
Cl^-	Proximal convoluted tubule	↓	(98)
	Tracheal epithelial cells	↑	(65)
	A6 kidney cells	↓	(64)
	Intestinal epithelial cells	↑	(99)
H^+	Turtle urinary bladder	↓	(68)
	Frog skin	↓	(69)
	Toad urinary bladder	↑	(70)

[a] Decrease in transport.
[b] Increase.

phospholipase A_2 activity (24). Additionally, toad urinary bladder has been shown to produce at least three PG, namely, PGI_2, $PGF_{2\alpha}$, and PGE_2 (70).

Over the last several years, there have been a number of reports indicating that PG can modify Na^+ transport in renal epithelia. A controversial issue has been whether the PG affects Na^+ transport in the kidney by a direct epithelial action or through secondary processes, such as changes in hemodynamics, since PG are known to decrease the glomerular ultrafiltration coefficient (77, 78) as well as cause renin release (79). However, more recent studies have demonstrated that the natriuretic effect of PGE_2 was a direct action on the tubular system. Using isolated rabbit cortical collecting tubules, Holt and Lechene (80) have been able to demonstrate that PGE_2 (10^{-5} *M*) could inhibit net ionic flux of Na^+ across the tubule and further; this inhibition could be partially reversed by meclofenamate, an inhibitor of PG synthesis. Furthermore, Grenier *et al.* (81) have shown that kinins in the rabbit collecting tubule are natriuretic and that this effect was brought about through the stimulation of PGE_2 formation by the tubular cells. Stokes and Kokko (82) have also demonstrated inhibition of Na^+ transport across this same tissue at a similar concentration of PGE_2. Kokko (83) has further suggested that this inhibitory action of PGE_2 on Na^+ transport may be mediated by a specific PG receptor located on the basal or blood side of the epithelial cell that may act to (i) decrease Na^+-K^+-ATPase activity or (ii) reduce the influx of Na^+ across the luminal membrane. Later studies by Culpepper and Andreoli (84) compared the interaction of PGE_2 with cholera toxin and forskolin, known stimulators of adenylcyclase, on modifying NaCl transport in the thick ascending limb of Henle in the mouse nephron. Their findings revealed that PGE_2 blocked the NaCl transport that was induced by cholera toxin and forskolin (increased adenylcyclase). These authors further suggested that PGE_2 reduced the rate of cAMP formation by interacting with the (Gi) inhibitory subunit of the adenylcyclase complex. Evidence consistently indicates that in the mammalian nephron PG inhibits Na^+ transport across the distal tubule with little or no effects on proximal tubule transport. This inhibition may be the result of a PGE receptor linked to adenylcyclase via inhibitory G proteins.

In contrast to the mammalian nephron, in the toad urinary bladder, another model renal epithelia, PG have been reported to increase Na^+ transport. Orloff *et al.* (85) were first to suggest that PGE_1 stimulated Na^+ transport in toad urinary bladder. Later studies by Lipson and Sharp (86) confirmed this finding by demonstrating that PGE_1 stimulated the short-circuit current (a measure of net Na^+ transport) across the toad bladder. They were also able to potentiate this PGE_1 stimulation of Na^+ current with theophylline. This suggested that the action of PGE_1 may be via activation and stimulation of adenylcyclase. Both of the studies found that PGE_1 was active in the bladder when on the serosal or blood side of the epithelium. Previous studies have also shown that PG, particularly PGE_2, produced dose-dependent effects on adenylcyclase, with high doses (10^{-6} *M*) stimulating and low doses (10^{-8} *M*) inhibiting adenylcyclase (87, 88). Halushka *et al.* (89) in a more recent study have provided evidence that prostaglandin PGI_2 also stimulated Na^+ reabsorption in toad bladder, thereby functioning as a antinatriuretic factor. In another amphibian tissue, the frog skin, similar results have been reported. Barry and Hall (90) were able to demonstrate stimulation of Na^+ transport across frog skin by serosal PGE_1 (0.5×10^{-6} *M*) and Fassina *et al.* (91) also showed that PGE_1 (10^{-6} *M*) on the serosal surface could stimulate short-circuit current across the frog skin. The reason for this apparent dif-

ference in response to Na^+ transport between the nephron and amphibian tissue may well be a species difference in the specific receptor subtypes or G proteins expressed. PGE_2 is the active compound in the mammalian nephron, while PGE_1 and PGE_2 are active in amphibian tissue.

There is also ample evidence to indicate that PG are involved in modifying or regulating H^+ secretion in renal epithelium. There is a dichotomy of results again with PG and control of H^+ secretion much like that discussed above with regard to Na^+ transport. Clearly it has been shown by Hays *et al.* (92) that PGE_2 decreases proton secretion by 12% in the rabbit medullary collecting duct. In addition, these same investigators have shown that the PG inhibitor indomethacin stimulates H^+ secretion. Their experiments further suggest that this influence by PG on nephron acidification may be mediated by changes in adenylcyclase. In a preliminary report, Ascer *et al.* (93) indicated that PGE_2 also inhibited proton secretion in the turtle bladder. The turtle bladder is an epithelial tissue used as a model epithelium for renal transport studies. However, a later study (94) has convincingly shown that cAMP had no effect on H^+ secretion per se, in turtle bladder, but rather appeared to stimulate HCO_3 secretion.

In frog skin, another model epithelium, it has recently been shown that $PGF_{2\alpha}$ (10^{-8} *M*) inhibited H^+ excretion in both normal skin and skin from frogs in metabolic acidosis (95). In these same studies the investigators were also able to stimulate H^+ excretion in normal skin by blocking PG synthesis with ibuprofen. Thus, in the amphibian integument PG appeared to contribute in regulating acid secretion and maintaining cell pH, similar to that shown for the rabbit cortical collecting tubule.

Contrary to the PG actions above in renal tubule, turtle bladder, and frog skin, Frazier and Yorio (70) have demonstrated stimulation of H^+ excretion by PGE_2 in toad urinary bladder. PGE_2 in this tissue was effective in stimulating H^+ excretion in the range of 10^{-5}–10^{-6} *M*. It was also shown in this study that metabolic acidosis, which stimulated H^+ excretion in this tissue, also stimulated the formation of PGE_2, as determined by high-pressure liquid chromatography. In addition, this study also revealed that during metabolic acidosis there was an increased incorporation of [^{3}H]-arachidonic acid into $PGF_{2\alpha}$, PGE_2, and 6-keto-$PGF_{1\alpha}$ from arachidonic acid. These authors concluded that (i) PGE_2 was an important mediator of H^+ excretion (ii) endogenous PGE_2 concentrations are increased in response to metabolic acidosis in the toad urinary bladder, and (iii) metabolic acidosis stimulates phospholipase A_2 activity in toad bladder, leading to an increased synthesis in PGE_2.

At the present time, there is overwhelming evidence to support the contention that PG derived from membrane phospholipids plays a crucial role as a second messenger in mediating and modifying Na^+ and H^+ transport in various epithelial tissues. An explanation for the varied differences observed in PG effects on the various transport systems, other than species difference, is not readily apparent at this time; however, it could reflect tissue selectivity with regard to the presence of PG receptor subtypes and/or G proteins in these tissues. Additional studies in this area are warranted to delineate the reason for the various differences in PG effects.

Summary

Our understanding of the role of phospholipids in ion transport processes is only beginning to be appreciated. Although the role of polyphosphoinositide and its derived second messenger molecules IP_3, diacylglycerol, and arachidonic acid are well studied, we are still not certain as to how changes in the lipid bilayer structure influence the status of ion channels. This review focused on those studies which show a strong correlation with ion conductance changes and the status of the membrane phospholipids. In addition, a number of observations point to a major role of lipid second messengers that activate enzymes involved in protein phosphorylations, i.e., protein kinase C, as major regulators of a variety of ion channels and transporters. Such lipid second messengers provide a cellular mechanism whereby hormones, neurotransmitters, and pharmacologic agents functionally control the ionic environment and intracellular pH of target cells. Some of these pathways still remain to be elucidated; however, an appreciation for the participation of membrane phospholipids in these actions has been presented.

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