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COMMENTARY

Lipid Metabolism as a Target for Potassium Channel Effectors

Adam Szewczyk and Slawomir Pikula†‡*

LABORATORIES OF *INTRACELLULAR ION CHANNELS & †LIPID METABOLISM, DEPARTMENT OF CELLULAR BIOCHEMISTRY, NENCKI INSTITUTE OF EXPERIMENTAL BIOLOGY, WARSAW, POLAND

ABSTRACT. K^+ channel effectors are widely used in the treatment of various diseases, including diabetes mellitus type II, hypertension, and cardiac arrhythmia. In addition, a constantly growing body of literature reveals that some of these substances, despite their direct effect on K^+ channels, may influence cellular lipid metabolism. As a result, membrane lipid content and cellular concentrations of lipid messengers are changed. Due to the dependence of K^+ channel activity on membrane lipids, these observations seem to be of particular importance not only to characterize secondary effects of K^+ channel effectors but also to understand the long-term effects of these agents on K^+ channel activity. BIOCHEM PHARMACOL **60**;5:607–614, 2000. © 2000 Elsevier Science Inc.

KEY WORDS. potassium channels; potassium channel inhibitors and openers; lipid metabolism; mitochondria; endoplasmic reticulum

Enormous progress has been made recently towards an understanding of the structure and function of K^+ channels. The accumulation of such knowledge helped scientists to realize that K^+ channels are potential targets for therapeutic intervention. First, specific K^+ channels have been functionally assessed to particular pathologies and diseases, among them some cardiac dysfunctions, diabetes mellitus type II, and hypertension [1–5]. Second, it was found that the primary cellular targets for many pharmaceuticals, such as antiarrhythmic drugs, antidiabetic sulfonylureas, and K^+ channel blockers and openers, are indeed various types of K^+ channels [6–12]. This was soon after the pharmacology of K^+ channels was established to a satisfactory extent, followed by synthesis of useful therapeutic agents. All known K^+ channel pore-forming subunits are integral membrane proteins. Hence, their activity is modulated not only by hydrophilic ligands but also by the lipid environment; for example, the sensitivity of K_{ATP} channels§ to ATP inhibition is antagonized by PIP and PIP_2 [13] acting on their $K_{ir}6.x$ subunits [14]. In addition, it has been shown that the phospholipid composition of the membrane affects the function of Ca^{2+}-activated K^+ channels from rabbit colon epithelium [15] and G protein-coupled K^+ channels from rat atrium [16]. Moreover, it has been observed that the Kv4.3 current, stably expressed in CHO cells, is blocked by polyunsaturated fatty acids [17], whereas the K^+ channels in retinal Muller glial cells [18] and neuronal two P-domain K^+ channels [19] are altered by arachidonic acid. A possible explanation of such an action of fatty acids is provided by the observation that Ca^{2+}-dependent K^+ channels in bovine adrenal chromaffin cells are modulated by lipoxygenase metabolites of arachidonic acid [20].

On the other hand, some modulators of the K^+ channels were found to influence lipid metabolism (for example PS synthesis by K^+ channel openers [21]) within the cell. The potency of K^+ channel inhibitors to affect cellular lipid metabolism may serve as a long-term feedback mechanism regulating cellular ion homeostasis. In this commentary, we would like to review results suggesting that pharmacological treatment targeted to K^+ channels can contribute to the effects of K^+ channels via modulation of cellular lipid metabolism (Fig. 1).

ACTIVATION OF PLC BY ANTIDIABETIC SULFONYLUREAS

Antidiabetic sulfonylureas such as glibenclamide and its derivatives are well-known inhibitors of plasma membrane K_{ATP} channels [22]. Inhibition of pancreatic β-cell K_{ATP} channels by sulfonylureas leads to an increased secretion of insulin by a mechanism that involves membrane depolarization, opening of voltage-regulated Ca^{2+} channels, Ca^{2+}

‡ Corresponding author: Dr. Slawomir Pikula, Nencki Institute of Experimental Biology, Department of Cellular Biochemistry, 3 Pasteur St., 02–093 Warsaw, Poland. Tel. (48) 22-659-8571, Ext. 347; FAX (48) 22-822-4352; E-mail: slawek@nencki.gov.pl

§ *Abbreviations*: BKCa, BK-type Ca^{2+}-activated K^+ channels; $[Ca^{2+}]_{in}$, intracellular Ca^{2+} concentration; CHO, Chinese hamster ovary; CYP, cytochrome P450; DAG, diacylglycerol; ER, endoplasmic reticulum; DCA, 1,12-dodecanedioic acid; GPI, glycosyl phosphatidylinositol; IP_1, inositol phosphate; IP_3, inositol 1,4,5-trisphosphate; K_{ATP} channels, ATP-dependent K^+ channels; $K_{ir}6.x$, inward rectifier K^+ channels; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIP, phosphatidylinositol-4-phosphate; PIP_2, phosphatidylinositol-4,5-bisphosphate; PLA_2, phospholipase A_2; PLC, phospholipase C; PS, phosphatidylserine; PSSI and PSSII, PS synthases I and II; TG, triacylglycerol; and TMB-8, [8-(*NN*-diethylamino)-octyl-3,4,5-trimethoxybenzoate].

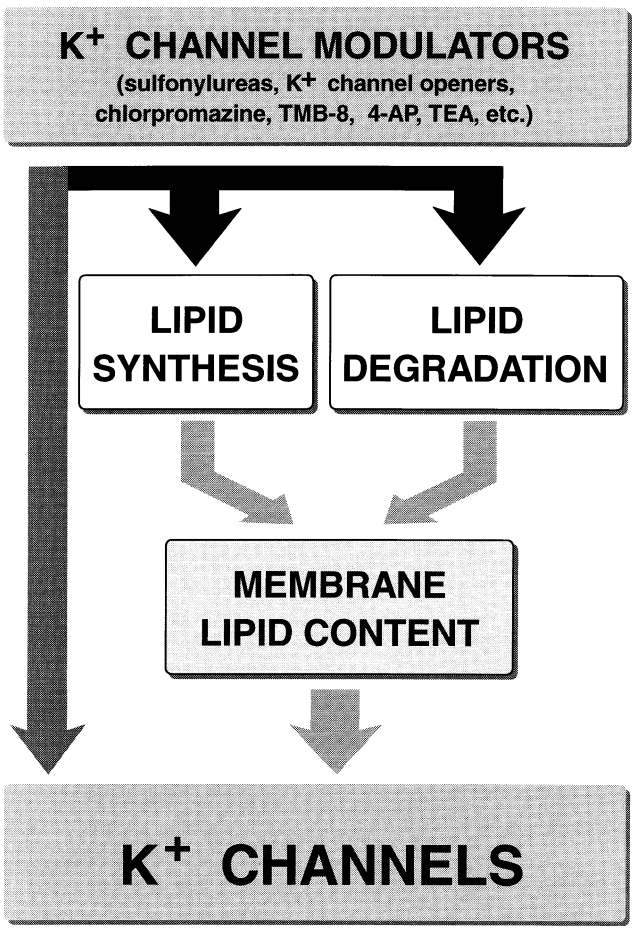

FIG. 1. Schematic representation of the interaction of K^+ channel effectors on lipid metabolism. This review article covers the pathways marked with black arrows. Abbreviations: TMB-8, [8-(*NN*-diethylamino)-octyl-3,4,5-trimethoxybenzoate]; 4-AP, 4-aminopyridine; and TEA, tetraethylammonium.

influx, and a rise in $[Ca^{2+}]_{in}$ from 10^{-8} to 10^{-5} M [23]. Despite these effects on insulin secretion, antidiabetic sulfonylureas exhibit pleiotropic extra-pancreatic action (for a review, see Ref. 24). For example, a sulfonylurea derivative, glimepiride, was found to interact with GPI lipids, forming membrane protein anchors in the adipocyte plasma membrane [25]. GPI lipids are enriched in caveolae, where a GPI-specific PLC, caveolin-1, a characteristic protein of caveolae, and acylated tyrosine kinases of the *src* family are also present [25]. Glimepiride and glibenclamide were found to stimulate the GPI-specific PLC activity and tyrosine phosphorylation of caveolin-1 through direct interaction with their respective enzymes. In accordance with the higher *in vivo* potency of glibenclamide than glimepiride and in the *in vitro* glucose transport/metabolism, the EC_{50} values for GPI-specific PLC activation and caveolin-1 phosphorylation were lower for glimepiride than for glibenclamide, although still higher than the reported therapeutic plasma concentrations of these drugs [25]. The stimulation of protein tyrosine phosphorylation by sulfonylureas does not involve the insulin signaling cascade and may be coupled to the activation of specific protein phosphatases regulating glucose transport and metabolism. In fact, it has been observed that the time-dependent accumulation of glimepiride in caveolae parallels the stimulation of glucose transport and cellular metabolism. Therefore, even at lower drug concentrations, this mechanism of sulfonylurea action might become effective *in vivo* due to the longer exposure times [25]. On the other hand, glibenclamide was found to elicit no effect on phospholipid synthesis in isolated guinea pig gastric glands [26], and this may suggest a tissue specificity for glibenclamide.

K^+ CHANNEL BLOCKERS AND LIPID SYNTHESIS

Despite the neuroleptic action of chlorpromazine, this drug affects the activity of various ion channels, including some K^+ channels. Chlorpromazine is a potent inhibitor of neuronal voltage-gated K^+ channels [27] and BKCa channels in the rat motor cortex [28]. In addition, chlorpromazine and related phenothiazines inhibit the K_{ATP} channels in HIT-T15 B-cells, as shown by using whole-cell current clamp, single channel recordings and measuring $^{86}Rb^+$ fluxes, with IC_{50} values in the low micromolar concentration range [29]. On the other hand, the drug activates chloride currents in *Xenopus* oocytes [30]. In human atrial myocytes, it also activates B-type Ca^{2+} channels [31], whereas it inhibits L-type Ca^{2+} channels in pheochromocytoma PC12 cells [32].

Studies on the synthesis of neutral lipids and phospholipids by primordial human placenta from [^{3}H]glycerol in the presence of chlorpromazine revealed a strong effect of this drug on lipid metabolism [33]. In the placenta, chlorpromazine evoked a 2-fold accumulation of [^{3}H]PA, an important intermediate molecule in phospholipid synthesis, followed by a 5- to 6-fold increase in [^{3}H]PI synthesis within the first 5–10 min of incubation. This resulted in a progressive PI and [^{3}H]DAG accumulation within the next 30 min. On the other hand, the synthesis of [^{3}H]TG and [^{3}H]PC was at the same time attenuated by 20 and 30%, respectively [33]. It has been suggested, therefore, that the chlorpromazine-induced increase in the concentrations of PI and DAG may interfere with signal transduction pathways in the placentae of pregnant patients treated with the drug [33]. Detailed studies on chlorpromazine effects on lipid metabolism in 8- to 10-week-old human placenta, utilizing [^{3}H]glucose as a substrate, revealed that the chlorpromazine stimulation of PA formation is accompanied by an inhibition of PA phosphohydrolase and DAG acyltransferase activities [34].

Using a different experimental model than the placenta, it has been reported that chlorpromazine interferes with the metabolism of inositol phospholipids in human platelets [35–37]. In these cells, after 10 min of incubation with [^{32}P]P_i, 25 μM chlorpromazine significantly increased the incorporation of radioactivity into PIP and PIP_2 [35]. On the basis of these results, it has been suggested that the drug

may affect kinase and phosphohydrolase reactions that maintain the steady-state level of PIP in the metabolically active pool of phosphoinositides, resulting in a 92% increase in the PIP level of this pool. Chlorpromazine also stimulates the turnover of phosphoinositides and probably the *de novo* synthesis of phosphoinositides and/or DAG formation through PLC and PLD activation [35].

TMB-8, another inhibitor of K_{ATP} channels [38], is also well known as a blocker of agonist-stimulated release of Ca^{2+} from intracellular stores. In addition, TMB-8 directly modulates ion channel activities. Using whole-cell current clamp, single-channel recordings and determination of $^{86}Rb^{+}$ fluxes, it has been shown that TMB-8 inhibits K_{ATP} channels in HIT-T15 B-cells under conditions where these channels are activated by ATP depletion or by their opener, diazoxide [38]. At concentrations of the drug (25–150 μM) that inhibit Ca^{2+} release from NIE-115 cells, TMB-8 was found to inhibit PC formation, whereas it stimulates the synthesis of PI (2-fold), PG (up to 40-fold!), and PS (3-fold) [39]. Moreover, it has been observed that TMB-8 does not inhibit phosphatidate phosphatase or enzymes responsible for the *de novo* synthesis of PC, but it is a competitive inhibitor (K_i = 10 μM) of a low-affinity (K_i = 20 μM) choline transport system in NIE-115 cells. TMB-8 concomitantly decreases cellular uptake of P_i and inositol but not that of ethanolamine or serine. These effects were Ca^{2+} independent [39]. In the case of PG, TMB-8 is suggested to specifically affect the synthesis of phospholipid from CDP-DAG in NIE-115 cells [39].

Other effectors of K^{+} channels, i.e. quinine, 4-aminopyridine, and tetraethylammonium, were also found to modify phospholipid metabolism in the human Jurkat T cell line, by inhibiting the synthesis of PC and PE, and influencing the high-affinity choline and/or ethanolamine transport systems in Jurkat T cells [40].

EFFECT OF K^{+} CHANNEL BLOCKERS ON PS SYNTHESIS

The amount of PS in mammalian cell membranes varies from 1 to 10 mol% of total phospholipids and is tissue specific [41]. This phospholipid is solely formed by a unique, enzyme-catalyzed, Ca^{2+}-dependent phospholipid base exchange reaction occurring in the ER membranes of mammalian cells [42–45]. It has been documented [46] that, in rat liver ER membranes, the specific stearoyl-polyunsaturated molecular species of PE and PC are preferentially converted to PS via this reaction. The studies performed on PS auxotrophic mutants of CHO cells revealed that two enzymes catalyzing the synthesis of PS are present in higher eukaryotes: PSSI, converting PC and PE to PS, and PSSII, which utilizes only PE [47–49]. Two enzymes also probably exist in rat hepatocytes with a similar specificity as in CHO cells [50, 51]. It is postulated that despite PS synthesis, the phospholipid base exchange reaction plays a major role in the interconversion of molecular species of phospholipids preexisting in the membranes (Fig. 2).

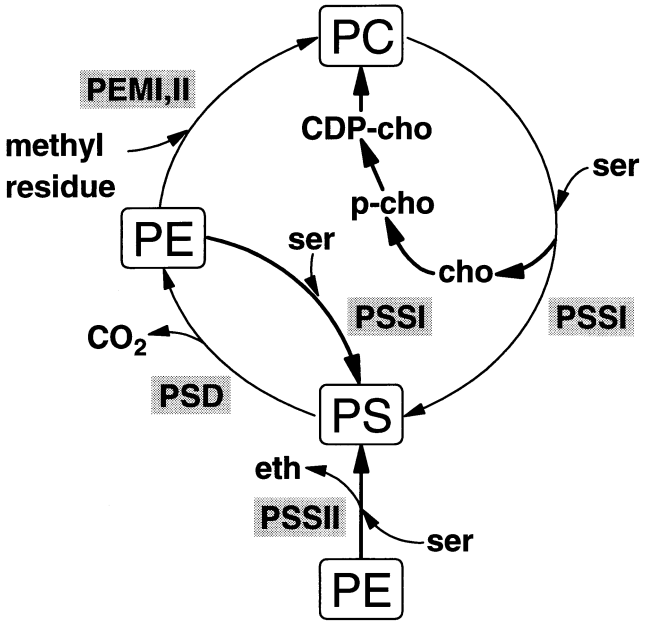

FIG. 2. Interconversion of major phospholipid classes in mammalian cells. PS is exclusively synthesized from PC/PE and serine (ser) via the phospholipid base reaction catalysed by PSSI and PSSII synthases located in ER membranes. eth = ethanolamine; cho = choline. PS is decarboxylated in mitochondria to PE by PSD. PE is methylated to PC by two methyltransferases: PEMI and PEMII. The major route of PE and PC synthesis is achieved via a *de novo* pathway involving multiple enzymes located in the cytosol and in ER membranes and multiple intermediate substrates; these substrates are shown for PC synthesis: choline, phosphocholine (p-cho), and CDP-choline (CDP-cho). For further details, please refer to *Biochim Biophys Acta* 1348, 1997 (section: Lipids and Lipid Metabolism), which is fully devoted to the phospholipid synthesis pathways.

In Jurkat T cells, one of the major targets of K^{+} channel blockers is PS synthesis. In these cells, it was found that quinine and quinidine, two K^{+} channel blockers, markedly enhance PS synthesis, which is accompanied by a significant decrease in the PC and PE synthesis rate due to the inhibition of uptake of PC and PE precursors [52], as also observed with cinchonine, cinchonidine, and chloroquine [52]. However, these latter three drugs were unable to stimulate PS synthesis, indicating that the effect of the K^{+} channel blockers quinine and quinidine on PS synthesis is specific [52]. Enhanced synthesis of PS in the presence of K^{+} channel blockers mimicked the effects of exogeneously added PS to Jurkat T cells, i.e. inhibition of interleukin-2 synthesis, probably due to a defect in DAG production [40].

In the same direction, the results of *in vitro* experiments revealed that a K_{ATP} channel blocker, the guanidine derivative U-37883A, but not its biologically inactive analog, U-42069 [53], is able to stimulate the synthesis of PS in ER membranes isolated from rat liver [21]. The effect of U-37883A was concentration dependent, with an EC_{50} of

54 μM [21]. The stimulation of PS synthesis was optimal under conditions when active transport of Ca^{2+} into the lumen of ER vesicles was induced, probably by the presence of the drug. Moreover, the effect of U-37883A was abolished by a calcium ionophore, A23187, and by thapsigargin, a specific inhibitor of Ca^{2+}-ATPase. U-37883A did not affect the affinity of the base exchange reaction for serine, but reduced the EC_{50} value of the enzyme for Ca^{2+} [21]. These observations suggest that U-37883A may indirectly stimulate PS synthesis by influencing Ca^{2+} homeostasis in hepatocytes (Fig. 3A).

Another indirect mechanism of action of K^+ channel blockers on PS synthesis was described for the imidazole antimycotics: miconazole, econazole, triclomazole, and α-naphthoflavone. These substances, known as inhibitors of CYPs and as K^+ channel blockers, were found to stimulate biosynthesis of PS in Jurkat T cells [54, 55]. The effect of the antimycotics was independent of Ca^{2+} influx and membrane potential changes [55]. Therefore, it has been postulated that the effect of these substances on PS synthesis is evoked through CYPs [55].

CYPs constitute a superfamily of hemoproteins that play a major role in the metabolism of endogenous compounds (steroid hormones, bile salts, and fatty acids) and xenobiotics [56]. Moreover, various CYPs are induced by these substances via transcriptional and posttranscriptional mechanisms [57]. Among many chemicals, clofibric acid, a xenobiotic compound used as a hypolipidemic drug, induces specific isoforms of CYP and is responsible for a variety of hepatic responses, such as a rapid proliferation of peroxisomes, smooth ER membranes and mitochondria, and alterations in lipid metabolism [58, 59]. In the case of the stimulation of PE synthesis via the base exchange reaction [50, 60, 61], these alterations are accompanied by the activation of microsomal ω-hydroxylation of fatty acids due to the induction of CYP isoenzymes [62], especially of the CYP4A1 isoform [60, 61]. This isoform particularly catalyzes the hydroxylation of lauric acid, which is a known inhibitor of phospholipid synthesis [63]. The ω-hydroxylation of lauric acid by CYP4A1 results in the formation of a primary alcohol, which is subsequently converted to a dicarboxylic acid, DCA. In response to pathophysiological conditions, such as diabetes mellitus, a high fat diet, or fasting, the level of DCA increases [64].

The results provided lead to the following possible mechanism. An increased amount of CYP4A1 protein requires specific molecular species of PE and PS for its optimal activity, which are provided through the activation of the phospholipid base exchange reaction. Moreover, the activation of CYP4A1 is responsible for lowering the concentration of lauric acid, the inhibitor of the base exchange reaction (Fig. 3B). This mechanism is corroborated by observations made after administration of a suicide inhibitor of CYP4A1, 10-undecynoic acid. When CYP4A1 is inhibited by 10-undecynoic acid, the level of lauric acid increases in hepatocytes, and phospholipid synthesis via the base exchange mechanism is inhibited [65].

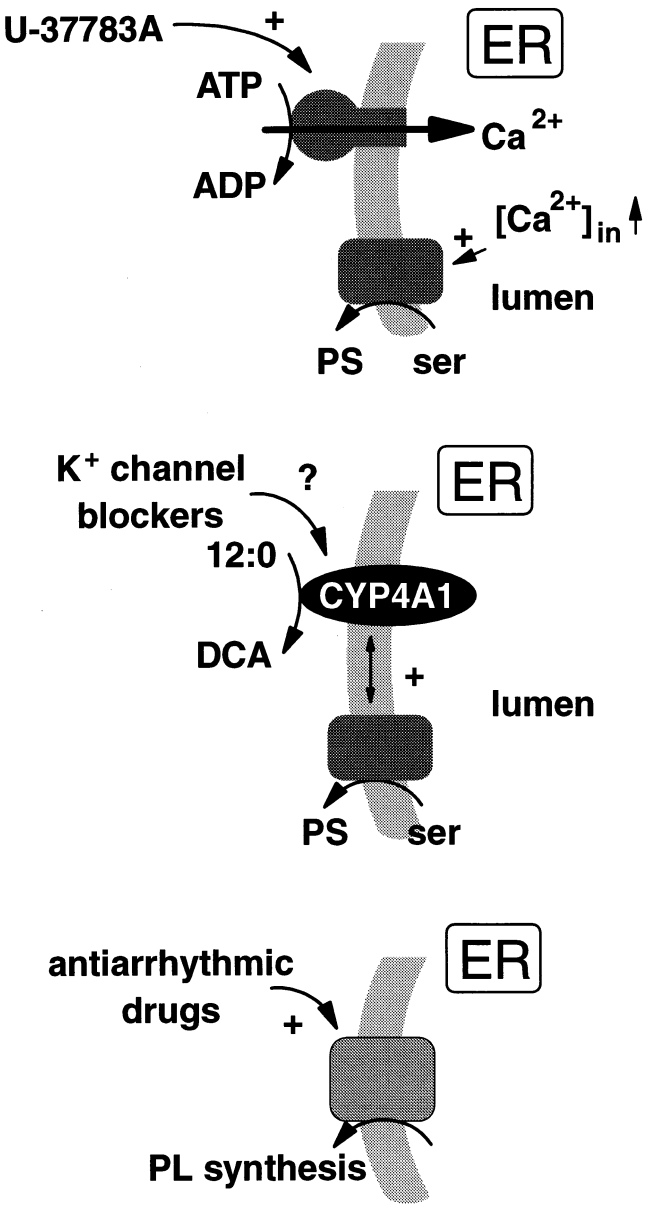

FIG. 3. Possible mechanisms of action of K^+ channel effectors on phospholipid (PL) synthesis. (A) The K^+ channel blocker U-37883A, through activation of Ca^{2+}-ATPase, may increase $[Ca^{2+}]$ within the lumen of ER membranes and, therefore, stimulate PS synthesis via the PL base exchange reaction. (B) The interrelation between CYP4A1 isoform activity and PE synthesis in rat liver ER membranes. CYP4A1 converts lauric acid (12:0), an inhibitor of PE synthesis, into harmless DCA. As a result, the rate of synthesis of PE via PL base exchange increases. In turn, the molecular species of PE synthesized in this reaction stimulate CYP4A1 activity. (C) The antiarrhythmic drugs may directly modulate the activity of some lipid synthesizing enzymes. Other explanations are within the text.

EFFECT OF ANTIARRHYTHMIC DRUGS ON LIPID SYNTHESIS

Antiarrhythmic drugs are among various K^+ channel effectors modulating phospholipid metabolism. Bretylium, clofilium, propranolol, *N*-acetylprocainamide, and amiodarone are able to decrease, in a concentration-dependent

manner, the biosynthesis of PC and PE in Jurkat T lymphocytes, impairing the uptake of choline and ethanolamine by these cells [66]. The efficiency of the drugs to inhibit phospholipid synthesis was in the following order of effectiveness: clofilium > amiodarone > propranolol = bretylium > *N*-acetylprocainamide, with IC_{50} values varying from 3 to 5 μM for clofilium, a voltage-gated K^+ channel blocker, to over 200 μM for *N*-acetylprocainamide. In addition, among the tested compounds, clofilium stimulated PS synthesis in these cells (EC_{50} = 50 μM) in a manner similar to other K^+ channel blockers: quinine, 4-aminopyridine, and tetraethylammonium [66].

The effect of antiarrhythmic drugs on the biosynthesis of phospholipids was also observed in isolated hamster hearts [67]. When these hearts were perfused with radiolabelled glycerol in the presence of methyllidocaine, the extent of labelling in PS, PI, DAG, CDP-DAG, and TG was enhanced, but not in PC and PE. In *in vitro* assays, it has been found that the enzymes involved in the synthesis of acidic phospholipids, i.e. phosphatidate phosphatase and CTP: phosphatidate cytidyltransferase, were stimulated [67]. Therefore, it has been postulated that the enhanced synthesis of neutral lipids and phospholipids in hearts perfused with methyllidocaine was due to the direct activation of the key enzymes of lipid metabolism by the drug (Fig. 3C).

K^+ CHANNEL OPENERS AND LIPID METABOLISM

K^+ channel openers are chemically distinct substances, but some of them as a common feature are able to activate K_{ATP} channels (for a review, see Ref. 68). Due to this action, they can modify the metabolism of inositol phospholipids [69]. Kil769, for example, acts intracellularly in vasorelaxation of the isolated porcine coronary artery, where it elicits some effects on the force of contraction, $[Ca^{2+}]_{in}$, and IP_1 formation. Both Kil769 (10 μM) and verapamil (1 μM), which produced submaximal relaxation, were found to reduce the increase in $[Ca^{2+}]_{in}$ and the force of contraction induced by 25 mM KCl; however, in contrast to verapamil, Kil769 reduced the force of contraction more strongly than $[Ca^{2+}]_{in}$. In addition, Kil769 inhibited IP_1 formation induced by a K^+ channel blocker, U46619, and glibenclamide reversed the inhibitory effect of Kil769 [69]. These results suggest that the opening of K^+ channels by Kil769 reduces the Ca^{2+} sensitivity of contractile elements and inositol phospholipid hydrolysis, which is related to the Ca^{2+} release from intracellular stores.

The effects of K^+ channel openers on inositol phospholipid hydrolysis were also studied in rabbit mesenteric artery [70]. The experimental model was as follows: the membrane hyperpolarization of smooth muscle cells was induced by pinacidil, a K^+ channel opener, and then the effects on Ca^{2+} mobilization induced by noradrenaline were investigated by measuring $[Ca^{2+}]_{in}$, isometric tension, membrane potential, and production of IP_3. Pinacidil (0.1 to 10 μM), in a concentration-dependent manner, hyperpolarized the plasma membrane and reduced its resistance. Glibenclamide (1 μM) blocked the effect of pinacidil [70]. Moreover, pinacidil (1 μM) inhibited depolarization of the membrane with associated oscillations caused by noradrenaline (10 μM), and glibenclamide (1 μM) prevented the action of pinacidil [70]. Noradrenaline (10 μM) also produced a large phasic and a subsequent small tonic increase in $[Ca^{2+}]_{in}$ with associated oscillations. These changes were coincident with phasic, tonic, and oscillatory contractions, respectively. Pinacidil (0.1 to 1 μM) inhibited the increases in $[Ca^{2+}]_{in}$ and in tension induced by noradrenaline, while glibenclamide reversed the effect of pinacidil [70]. Moreover, pinacidil (1 μM) inhibited the contraction induced by 10 μM noradrenaline in mesenteric artery strips treated with A23187 (which functionally empties intracellular Ca^{2+} stores), suggesting that membrane hyperpolarization inhibits Ca^{2+} fluxes activated by noradrenaline. In the presence of 2 mM EGTA, noradrenaline (10 μM) transiently increased $[Ca^{2+}]_{in}$, tension, and synthesis of IP_3. These effects were inhibited by pinacidil (above 0.1 μM) and were sensitive to intracellular K^+ concentration. The inhibitory effects of pinacidil were reversed by glibenclamide (1 μM) [70]. These results suggest that pinacidil inhibits noradrenaline-induced Ca^{2+} release from intracellular stores through an inhibition of IP_3 synthesis by evoking membrane hyperpolarization [70].

Recently, it has also been shown that KRN4884, a novel pyridinecarboxamidine-derived K^+ channel opener, affects lipid metabolism in rats [71, 72].

K^+ CHANNEL EFFECTORS VERSUS PLA_2

Quinacrine, a PLA_2 inhibitor, is able to inhibit intracellular glibenclamide-sensitive K^+ channels in follicle-enclosed *Xenopus* oocytes [73]. Moreover, it is postulated that some general anesthetics that inhibit BKCa channels at clinically relevant concentrations produce their effects by disrupting the arachidonic acid signal transduction pathway [74]. In addition, the treatment of excised patches with exogenous arachidonic acid results in a 4-fold increase in BKCa channel activity. Subsequent exposure of these patches to concentrations of halothane (0.6 mM), ketamine (100 μM), or etomidate (10 μM) that would block the channel by 60–80% in the absence of arachidonic acid did not reduce the channel activity. Arachidonic acid treatment resulted in an increase in EC_{50} for ketamine from 3.4 to 693 μM [73]. It has also been found that PLA_2 inhibitors, quinacrine (1 μM), aristolochic acid (250 μM), and octadecylbenzoylacrylic acid (7 μM), inhibited BKCa channels in a manner indistinguishable from general anesthetics. Moreover, aristolochic acid and ketamine inhibited the PLA_2-mediated production of arachidonic acid in GH3 cells [73]. These results point to the interpretation that some K^+ channel effectors may interfere with the activity of cellular PLA_2s.

CONCLUDING REMARKS

To date, hundreds of chemical substances are known to affect various K^+ channel activities. Moreover, K^+ channels, as integral membrane proteins, are natural targets for lipids and lipid-derived substances. In this commentary, we have reviewed overlapping areas: the modulation of lipid metabolism by K^+ channel effectors and their effects on the permeability of membranes to K^+. We believe that this kind of double action has to be considered as an important parameter in assessing long-term effects of drugs used in therapies targeting K^+ channels. On the other hand, it is possible that lipid metabolism modulators could be used in the future to create a long-term regulatory feedback mechanism acting on K^+ channel activity.

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