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STUDIES ON THE MECHANISM OF ALTERATION BY PROPRANOLOL AND MEPACRINE OF THE METABOLISM OF PHOSPHOINOSITIDES AND OTHER GLYCEROLIPIDS IN THE RABBIT IRIS MUSCLE

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Abstract—We have investigated the effects and mechanism of action of propranolol and mepacrine, two drugs with local anesthetic-like properties, on phospholipid metabolism in rabbit iris and iris microsomal and soluble fractions. In the iris, propranolol, like mepacrine [A. A. Abdel-Latif and J. P. Smith, *Biochim. biophys. Acta* **711**, 478 (1982)], stimulated the incorporation of [^{14}C]arachidonic acid ([^{14}C]AA) into phosphatidic acid (PA), CDP-diacylglycerol (CDP-DG), phosphatidylinositol (PI), the polyphosphoinositides (poly PI) and DG, and it inhibited that of phosphatidylcholine (PC), phosphatidylethanolamine (PE), triacylglycerol (TG) and the prostaglandins. Similarly, mepacrine, like propranolol [A. A. Abdel-Latif and J. P. Smith, *Biochem. Pharmac.* **25**, 1697 (1976)], altered the incorporation of [^{14}C]oleic acid, [^{3}H]glycerol, ^{32}Pi and [^{14}C]choline into glycerolipids of the iris. Time–course studies in iris muscle prelabeled with [^{14}C]AA showed an initial decrease in the production of DG and a corresponding increase in that of PA by the drugs, followed by an increase in accumulation of DG at longer time intervals (60–90 min). The above findings are in accord with the hypothesis that these drugs redirect glycerolipid synthesis by inhibiting PA phosphohydrolase. Propranolol and mepacrine stimulated the activities of DG kinase and phosphoinositide kinases and inhibited that of DG cholinephosphotransferase. The drugs had little effect on the activity of DG acyltransferase. It is concluded that propranolol and mepacrine redirect glycerolipid metabolism in the iris by exerting multiple effects on the enzymes involved in phospholipid biosynthesis. We suggest that these drugs could exert their local anesthetic-like effects by effecting an increase in the synthesis of the acidic phospholipids (PA, PI and the poly PI) and subsequently the binding of Ca^{2+} to the cell plasma membrane.

A number of reports have appeared in the past several years showing that a large variety of cationic amphiphilic drugs, local anesthetics and drugs with local anesthetic-like properties [1–11], including chlorpromazine [1], morphine [2, 3], fenfluramine and other cationic amphiphilic drugs [4], local anesthetics such as dibucaine [5], propranolol [5–10], and mepacrine [11], markedly altered glycerolipid metabolism in a wide variety of tissues. The overall effects of these drugs are to stimulate the *de novo* synthesis of CDP-diacylglycerol (CDP-DG*) and the acidic phospholipids, phosphatidic acid (PA), the phosphoinositides and cardiolipin and to depress the synthesis of triacylglycerol (TG), phosphatidylcholine (PC) and phosphatidylethanolamine (PE). It has been suggested that the rate of conversion to DG, by PA-phosphohydrolase, is inhibited by the drugs [4, 5]. This redirects glycerolipid synthesis away from the production of PC, PE and TG and toward the production of phosphoinositides and cardiolipin.

In previous communications from this laboratory [6, 7], we reported that in the rabbit iris muscle propranolol stimulated the biosynthesis of the acidic phospholipids and inhibited that of PC, PE and TG. In addition, this drug increased the biosynthesis of phosphatidylserine by stimulating the Ca^{2+}-dependent base-exchange reaction [6, 7]. During more recent studies on the incorporation of [1-^{14}C]arachidonic acid (AA) into glycerolipids and its conversion into prostaglandins by rabbit iris, we observed that mepacrine, a phospholipase-A_2 inhibitor, stimulated the labeling of the acidic phospholipids and DG and inhibited that of PC, PE and TG, an action similar to that of a cationic amphiphilic drug [11]. The finding that mepacrine increased the labeling of DG suggested to us that this drug could act on other metabolic pathways of phospholipid metabolism. These observations prompted us to reinvestigate the effects of propranolol and mepacrine on glycerolipids and their enzymes in the rabbit iris. This report describes the results obtained from these studies.

MATERIALS AND METHODS

Materials

[1-^{14}C]Arachidonic acid (sp. act. 56.5 mCi/mmole) was purchased from the Amersham Corp., Arlington Heights, IL; [1-^{14}C]oleic acid (sp. act. 40–60 mCi/mmole), [*methyl*-^{14}C]choline chloride (sp. act.

* Abbreviations: DG, diacylglycerol; PA, phosphatidic acid; PI, phosphatidylinositol; PI-P, phosphatidylinositol-4-P; PI-bis P, phosphatidylinositol-4,5-bisphosphate; poly PI, polyphosphoinositides; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TG, triacyglycerol; and AA, arachidonic acid.

55.0 mCi/mmole), [*oleoyl*-1-^{14}C]coenzyme A (sp. act. 57.8 mCi/mmole), [*methyl*-^{14}C]cytidine diphosphocholine (sp. act. 52.5 mCi/mmole), [2-^{3}H]glycerol (sp. act. 10.0 Ci/mmole), and [^{32}P]phosphorus, carrier free, were purchased from the New England Nuclear Corp., Boston, MA; [γ-^{32}P]adenosine-5-triphosphate (sp. act. 25 Ci/mmole) was obtained from ICN Chemical and Radioisotope Division, Irvine, CA. DL-Propranolol and L-α-phosphatidic acid (sodium salt) were purchased from the Sigma Chemical Co., St. Louis, MO. Phospholipids, including polyphosphoinositides, and neutral lipids were from sources described previously [12, 13]. All other chemicals were of reagent grade.

Methods

Preparation and incubation of iris muscle. Albino rabbit eyes were obtained fresh either from a nearby slaughterhouse or from Pel-Freez Biologicals, Inc., Rogers, AR. The eyes were removed shortly after killing and were transported to the laboratory packed in ice. The iris was removed from the eye and placed in Krebs–Ringer bicarbonate buffer (pH 7.4).

In general, two irises from pairs were incubated (of the pair one was used as control) in 1 ml of the isoosmotic medium that contained 0.25 μCi of [1-^{14}C]arachidonic acid (or [1-^{14}C]oleic acid) bound to 0.1 mg albumin, or 30 μCi ^{32}Pi, 32 μCi [^{3}H]glycerol, or 2 μCi [^{14}C]choline, as indicated, at 37° for 1 hr. Mepacrine and propranolol were added as indicated. At the end of incubation, the tissue was analyzed for glycerolipids.

Preparation of subcellular fractions. Methods of homogenization, subcellular fractionations and monitoring of the purity of the preparations by means of electron microscopy and enzyme markers were essentially as previously reported [6, 13]. Briefly, rabbit irises were homogenized in 10 vol. of 0.25 M sucrose containing 1 mM EDTA (pH 7.4) for 4 × 30 sec using a Super Dispax Tissumizer model SDT-182 (Takmar Co.) at maximum speed. The homogenate was centrifuged at 600 *g* for 10 min, and the nuclear pellet was discarded. The supernatant fraction was centrifuged at 10,000 *g* for 30 min, and the mitochondrial pellet was discarded. The supernatant fraction obtained was centrifuged at 110,000 *g* for 90 min to give a microsomal pellet and soluble fraction. The microsomal fraction was retained as indicated, and the soluble fraction was fractionated with $(NH_4)_2SO_4$ according to the procedure of Thompson [14]. The precipitates obtained between 0 and 30% and between 30 and 50% saturation were dissolved in 50 mM imidazole buffer (pH 6.5) and dialyzed overnight against the same buffer. The microsomal and the 30 and 50% dialyzed $(NH_4)_2SO_4$ fractions were used in the following studies on the effects of mepacrine and propranolol on various enzymes of lipid metabolism.

The procedure used to prepare microsomal fractions from rabbit liver and brain was identical to the one described for iris muscle, except that a teflon homogenizer was used for tissue homogenization.

Assay of microsomal DG cholinephosphotransferase. Cholinephosphotransferase was assayed in microsomal fractions from rabbit iris muscle, liver and brain by measuring the rate of incorporation of phosphoryl[$^{14}CH_3$]choline into PC from CDP[$^{14}CH_3$]choline [15].

Assay of microsomal DG acyltransferase. DG acyltransferase in rabbit iris microsomes was assayed by measuring the incorporation of [^{14}C]oleic acid into TG from [*oleoyl*-1-^{14}C]oleoyl coenzyme A using both endogenous and exogenous diacylglycerols as substrates [16]. Formation of triacylglycerol was determined by means of one-dimensional thin-layer chromatography (TLC) (see below).

Assay of soluble PI phosphodiesterase. PI phosphodiesterase was assayed in the 30–50% $(NH_4)_2SO_4$ precipitate of the iris soluble fraction as described previously [13] in which the release of inositol phosphate was taken as a measure of the phosphodiesterase activity.

Assay of 1,2-DG kinase, PI kinase and PI-P kinase in the iris soluble fraction. During our studies on poly PI synthesis in the subcellular fractions of the iris muscle, we found that the 1,2-DG kinase and PI kinase are enriched in the 30% $(NH_4)_2SO_4$ fraction and PI-P kinase is enriched in the 30–50% $(NH_4)_2SO_4$ fraction; thus in the present study the activities of these enzymes were assayed in these fractions. The activities of DG-, PI- and PI-P kinases were determined by measuring the incorporation of ^{32}P from [γ-^{32}P]ATP into PA, PI-P and PI-bis P respectively.

The basic system for the assay of these enzymes (final volume 50 μl) contained: 50 mM imidazole buffer (pH 6.5), 10 mM $MgCl_2$, 0.2 mM [γ-^{32}P]ATP (sp. act. 2 Ci/mmole) and 50 μg protein. Incubations for DG kinase and PI-P kinase also contained 0.1 mM 1,2-dioleoylglycerol and 1 mM PI-P respectively. The reaction was started by the addition of [γ-^{32}P]ATP and incubation continued for 2 min at room temperature. The reaction was terminated with the addition of 1 ml of ice-cold 5% trichloroacetic acid (TCA) that contained 5 mM ATP. The lipids were extracted from the pellet and analyzed by means of thin-layer chromatography (see below).

Extraction and isolation of glycerolipids and prostaglandins. Glycerolipids were extracted twice from the iris with chloroform–methanol–HCl (400:200:1.5, by vol.) and phospholipids and neutral lipids were separated according to the method of Abdel-Latif *et al.* [12] and Snyder and Piantadosi [17] respectively. An aliquot of the lipid extract, equivalent to 80% of the total, was used for phospholipid analysis and the remainder was used for neutral lipid analysis. Phospholipids were separated by means of two-dimensional TLC with silica gel H, and their radioactive contents were determined as described previously [12]. Neutral lipids were separated by means of one-dimensional TLC with Whatman precoated silica gel LK6DF plates in a solvent system [17] of hexane–diethyl ether–acetic acid (80:20:1, by vol.). The neutral lipid spots were localized with autoradiography, and their radioactive contents were determined. Prostaglandins were extracted from the medium and separated by TLC as described previously [11].

In the studies on the subcellular fractions, the neutral lipids were extracted with chloroform–methanol (2:1, v/v) while the poly PI were extracted with chloroform–methanol–HCl (400:200;1.5, by vol.). PA and the poly PI were then chromatographed on

silica gel H precoated plates in a solvent system of chloroform–methanol–4 N NH_4OH (9:7:2.1, by vol.). PA and the poly PI spots were localized by exposure to I_2 vapor and by autoradiography. The phospholipid spots were scraped off the plates and their radioactive contents were determined.

Protein was estimated by the method of Lowry *et al.* [18]. The effects of mepacrine and propranolol on the incorporation of radioactivity into muscle glycerolipids or on the specific activities of the various enzymes of glycerolipid metabolism were expressed as percent of controls (in the absence of the drugs). All experiments were carried out in duplicate or triplicate.

RESULTS

Effects of propranolol on [1-^{14}C]AA metabolism by iris muscle

As with the effects of mepacrine on the incorporation of [1-^{14}C]AA into iris glycerolipids [11], propranolol stimulated appreciably the incorporation of this polyunsaturated fatty acid into acidic phospholipids and DG and inhibited that of PC, PE, TG and prostaglandin E_2 in a dose-dependent manner (Table 1). Thus, the maximal increases in the labeling of PA, PI, poly PI and DG were 660, 530, 161 and 170%, respectively, and the maximal decreases in those of PC and TG were 70 and 78%, respectively. As with mepacrine [11], propranolol inhibited the formation of prostaglandin E_2. It is interesting to note that the total radioactivity recovered remained almost constant at all concentrations investigated (75,848 cpm in the control and 72,306 cpm in the presence of 0.3 mM propranolol); however, under the same experimental conditions the radioactivity in the acidic phospholipids increased from 8,478 cpm to 41,478, or a 4-fold increase, and that in PC, PE and the neutral lipids decreased from 67,370 cpm to 30,828, or a 54% decrease (Table 1). These data indicate that in the intact tissue propranolol acts probably by inhibiting PA phosphohydrolase.

Time–course study on the effect of mepacrine on glycerolipid metabolism by rabbit iris prelabeled with [1-^{14}C]AA

The objective of this experiment was to explain the finding of an increase in DG when the cationic amphiphilic drugs are added to the incubation medium (Table 1). Time–course studies on the effect of mepacrine on glycerolipid metabolism in iris prelabeled with [1-^{14}C]AA revealed the following: (a) there was an initial increase in PA radioactivity in the presence of the drug, being maximal at about 2.5 min of incubation, and a corresponding decrease in that of DG (Fig. 1) and (b) the radioactivities in DG, and subsequently TG and PC, dropped significantly (by about 38%) during the first 15 min of incubation: then the radioactivity in DG increased by 40% after 90 min of incubation. PI labeling decreased with time of incubation. The initial decrease in DG radioactivity could be due to inhibition by the drug of PA phosphohydrolase. The increase in DG radioactivity with time was probably due to the multiple effects of the drug on the enzymes of glycerolipid metabolism (see below).

Effects of mepacrine on incorporation of radioactive lipid precursors by iris muscle

To show further that mepacrine does act as a cationic amphiphilic drug on glycerolipid metabolism, we investigated its effects on the incorporation of $^{32}P_i$, [^{3}H]glycerol, [^{14}C]oleic acid and [^{14}C]choline into glycerolipids of the rabbit iris (Table 2). The drug stimulated incorporation of the various lipid precursors into acidic phospholipids, CDP-DG and DG and inhibited the labeling of PC, PE and TG.

It can be concluded from the above studies, first,

Table 1. Effects of different concentrations of propranolol on [1-^{14}C]arachidonic acid incorporation into glycerolipids and its conversion into prostaglandins by rabbit iris*

		Effect of propranolol (mM) on incorporation of radioactivity (% of control)			
	(cpm/2 irises)	0.05	0.1	0.2	0.3
Tissue					
PA	491	131	290	530	760
CDP-DG	557	173	168	218	309
PI	4,508	181	306	533	630
Poly PI†	2,922	154	175	247	261
PC	18,593	73	69	56	30
PE	3,467	74	68	55	45
DG	5,533	158	173	240	270
TG	39,777	84	59	25	22
Medium					
Prostaglandin E_2	28,000	87	81	22	25

* In these experiments, two irises from pairs were incubated in 1 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) that contained 0.25 μCi of [1-^{14}C]arachidonic acid (sp. act 56.6 mCi/mmole) bound to 0.1 mg albumin at 37° for 1 hr in the absence and presence of various concentrations of propranolol. At the end of incubation, the tissue was analyzed for phospholipids and neutral lipids, and the medium was analyzed for prostaglandins as described in Methods.

† Phosphatidylinositol-4-Phosphate plus phosphatidylinositol-4,5-bisphosphate.

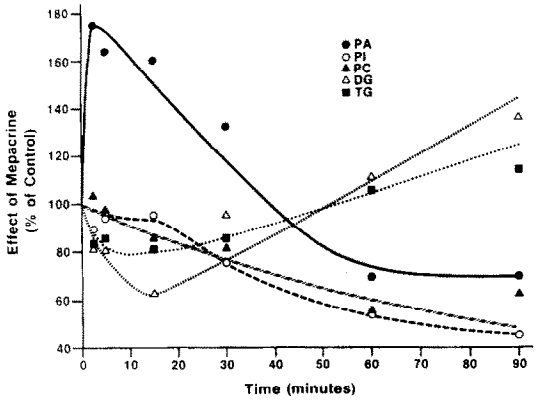

Fig. 1. Effect of mepacrine on glycerolipid metabolism by rabbit iris prelabeled with [1-^{14}C]AA. Key: PA (●); PI (○); PC (▲); DG (△); and TG (■). Irises were incubated (one of the pair was used as control, the other as experimental) in 1 ml of Krebs–Ringer buffer (pH 7.4) that contained 0.25 μCi of [1-^{14}C]AA bound to 0.1 mg albumin for 1 hr, at 37°. After incubation, the prelabeled irises were washed twice with nonradioactive buffer that contained 1 mg/ml bovine serum albumin and then were transferred into tubes that contained 1 ml of nonradioactive buffer. Incubations were then carried out in the absence and presence of 0.2 mM mepacrine at 37° for various time intervals. Phospholipids and neutral lipids were analyzed as described under Methods. All values are means of two separate experiments.

that mepacrine, like propranolol, had a cationic amphiphilic effect on glycerolipid synthesis in the iris and, second, that both drugs stimulated the accumulation of DG at long time intervals (Ref. 11, Tables 1 and 2).

Effects of mepacrine and propranolol on the activities of various enzymes of glycerolipid metabolism

The effects of these drugs on the activities of other enzymes of glycerolipid metabolism are summarized in Table 3. Mepacrine and propranolol stimulated the activity of DG kinase by 31 and 95% respectively. The drugs also stimulated the activities of the phosphoinositide kinases by 186–314% of their respective controls (Table 3). Of the other enzymes investigated, the drugs inhibited by up to 21% the activity of DG cholinephosphotransferase, but had a negligible effect on those of DG acyltransferase and PI phosphodiesterase (Table 3). Further studies on the effects of various concentrations of mepacrine and propranolol on the activity of cholinephosphotransferase in microsomal fractions from rabbit iris showed a dose-related inhibition of this enzyme by the drugs (Fig. 2). Similar data were obtained in microsomal fractions from rabbit liver and brain (data not shown).

DISCUSSION

We have demonstrated that in the rabbit iris (a) propranolol, like mepacrine [11], stimulated the incorporation of [^{14}C]AA into PA, CDP-DG, PI, the poly PI and DG, and inhibited that of PC, PE, TG and the prostaglandins (Table 1) and (b) mepacrine, like propranolol [6], stimulated the incopor-

Table 2. Effect of mepacrine on the incorporation of radioactive lipid precursors into glycerolipids of the rabbit iris*

	Effect of mepacrine on incorporation of radioactivity (cpm)											
	[^{32}P$_i$] Orthophosphate			[^{3}H]Glycerol			[^{14}C]Oleic acid			[^{14}C]Choline		
	Control	+Drug	% of Control	Control	+Drug	% of Control	Control	+Drug	% of Control	Control	+Drug	% of Control
PA	7,989	29,000	363	2,470	5,261	213	445	841	189			
CDP-DG	3,974	9,418	237	957	3,273	342	—†	—				
PI	22,826	44,054	193	5,195	15,377	296	794	1,135	143			
Poly PI	122,104	172,167	141	3,448	9,482	275	—	—				
PC	41,128	6,580	16	31,192	11,853	38	7,762	3,027	39	109,968	45,420	41
PE	7,935	3,015	38	6,643	4,650	70	1,624	861	53			
DG				28,082	40,157	143	3,906	5,664	145			
TG				47,821	31,084	65	9,054	3,622	40			

* Conditions of incubation were the same as described under Table 1 except that mepacrine (0.2 mM) was employed and the medium contained (μCi/ml): ^{32}P$_i$, 30; [^{3}H]glycerol, 32; [^{14}C]oleic acid, 0.25 and [^{14}C]choline, 1.
† Not determined.

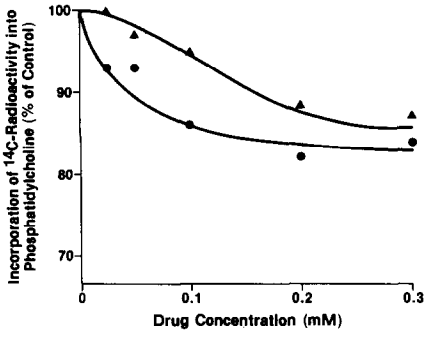

Fig. 2. Inhibition of DG cholinephosphotransferase of rabbit iris microsomes by mepacrine (●) and propranolol (▲). Incubation conditions are the same as in Methods.

ation of various lipid precursors into the acidic phospholipids and DG and inhibited that of PC and TG (Table 2). These data demonstrate that mepacrine, like propranolol, redirects phospholipid metabolism, an action reminiscent of a cationic amphiphilic drug, in that its net effect is to decrease the synthesis of PC, PE and TG and increase that of PA and the phosphoinositides. Mepacrine has been shown to block the release of fatty acids from phospholipids, a measure of phospholipase A_2 activity, in a variety of tissues [19]. Although it has been known that this drug has other effects, these authors assumed that its ability to block cellular responses is due to inhibition of phospholipase A_2 activity. During our studies on the metabolism of [^{14}C]AA by rabbit iris, we observed multiple effects of mepacrine on glycerolipid labeling and suggested that this drug could act as a cationic amphiphilic drug [11]. The data presented in this communication supported this conclusion and, furthermore, are in accord with the hypothesis that these drugs act by inhibiting PA phosphohydrolase [3–10].

The finding that both mepacrine and propranolol increased the labeling of DG from [^{14}C]AA, [^{14}C]oleic acid and [^{3}H]glycerol (Tables 1 and 2 and Ref. 11) is in accord with the work of Ilincheta de Boschero and Bazan [20] who reported that propranolol and phentolamine increased the incorporation of [^{14}C]glycerol and [^{14}C]palmitic into DG of bovine retina. We felt that the observations in the retina and iris were intriguing since they suggested that these drugs may affect other enzymes of glycerolipid metabolism. To explain this finding: (a) we carried out time–course studies on the effect of mepacrine on phospholipid metabolism in iris muscle prelabeled with [^{14}C]AA, and (b) we investigated the effects of mepacrine and propranolol on the enzymes of glycerolipid metabolism. The data presented in Tables 1 and 2 and Fig. 1 clearly indicate that in this tissue these drugs act by inhibiting PA phosphohydrolase; first, at short time intervals there is inhibition of DG formation and a corresponding increase in phosphatidate, and then there is accumulation of DG at longer time intervals (Fig. 1). In more recent studies, we found that propranolol inhibits the iris microsomal and soluble phosphatidate phosphohydrolase (A. A. Abdel-Latif and J. P. Smith, unpublished work). Brindley and Bowley

Table 3. Effects of mepacrine and propranolol on the activities of other enzymes involved in glycerolipid metabolism in rabbit iris*

Enzyme	Subcellular fraction	No. of determinations	Specific activity of enzyme [nmoles·(mg protein)$^{-1}$·hr^{-1}]		
			Control	Mepacrine (0.2 mM)†	Propranolol (0.2 mM)†
DG kinase	30% $(NH_4)_2SO_4$	6	0.26 ± 0.01	0.34 ± 0.01‡	0.51 ± 0,01‡
PI kinase	30% $(NH_4)_2SO_4$	6	0.21 ± 0.01	0.87 ± 0.05‡	0.72 ± 0.15‡
PI-P kinase	50% $(NH_4)_2SO_4$	8	0.49 ± 0.11	1.40 ± 0.20‡	1.74 ± 0.01‡
PI phosphodiesterase	50% $(NH_4)_2SO_4$	7	3430 ± 40	3940 ± 40	3330 ± 140
DG choline phosphotransferase	Microsomal	15	12.1 ± 0.1	9.6 ± 0.3§	10.6 ± 0.1§
DG acyltransferase	Microsomal	15	4.5 ± 0.1	4.4 ± 0.1	4.2 ± 0.1

* Enzymes were assayed as described in Methods. Results are expressed as mean ± S.E.M.
† Comparable effects were obtained with 0.1 mM concentrations of the drugs.
‡ Significant stimulation of enzyme activity as compared to the control ($P < 0.01$).
§ Significant inhibition of enzyme activity as compared to the control ($P < 0.05$).

[4] and Eichberg *et al.* [9] reported an inhibitory effect of cationic amphiphilic drugs on liver PA phosphohydrolase. Both of these authors employed membrane-bound [^{14}C]PA as substrate for PA phosphohydrolase [4, 9]. Accumulation of DG could result from the breakdown of phosphoinositides and other glycerolipids, as this tissue is enriched with phosphoinositide phosphodiesterases [13, 21].

Further studies on the effects of propranolol and mepacrine on the enzymes of phospholipid metabolism showed significant stimulation of DG kinase and phosphoinositide kinases (Table 3) and inhibition of choline phosphotransferase (Fig. 2). Cholinephosphotransferase is an enzyme that occurs at the DG branch point of phospholipid and TG biosynthesis. Thus, the 21% inhibition of cholinephosphotransferase observed in the present work, coupled with the stimulation of DG kinase and phosphoinositide kinases, by the drugs, could redirect glycerolipid synthesis away from PC, PE and TG and towards synthesis of PA, PI and the poly PI. Mepacrine and propranolol had a negligible effect on the activities of PI phosphodiesterase (Table 3). Lapetina *et al.* [22], working with horse platelets, also reported no effect of mepacrine on PI phosphodiesterase. Cationic amphiphilic drugs have been shown recently to increase ^{32}P-labeling of poly PI in rat cerebral cortex mince [23].

Mepacrine and propranolol had no effect on the activities of DG acyltransferase (Table 3). Brindley *et al.* [24] also reported that fenfluramine and other cationic amphiphilic drugs had no inhibitory effect against liver DG acyltransferase. The problem arises as to how the labeling of TG in the tissue is decreased by the drugs? The data presented above indicate that inhibition of PA phosphohydrolase and choline phosphotransferase, coupled with stimulation of DG kinase and the phosphoinositide kinase by the drugs, could redirect phospholipid synthesis towards phosphoinositides and away from TG, PC and PE. The stimulatory effects of cationic amphiphilic drugs on PA cytidylyltransferase [24] and on the formation of CDP-DG (Refs. 6 and 9 and Tables 1 and 2 of the present study) are in accord with this conclusion.

Thus in the present study we have demonstrated the following (summarized in Fig. 3): (a) in iris prelabeled with [^{14}C]arachidonic acid there is an initial decrease in the production of DG by the drugs and a corresponding increase in that of PA, thus suggesting an inhibition of PA phosphohydrolase. This conclusion is supported by the finding that propranolol inhibits both liver [9] and iris (A. A. Abdel-Latif and J. P. Smith, unpublished work) PA phosphohydrolase. (b) The drugs stimulated the activities of DG kinase and phosphoinositide kinases, inhibited that of choline phosphotransferase, and had negligible effects on the activities of PI phosphodiesterase and DG-acyltransferase. Thus we conclude that the multiple effects of propranolol and mepacrine on these enzymes could underlie the decrease in the flux of DG into PC, PE and TG, and the corresponding increase in that of PA and the phosphoinositides observed in the iris. Many of the effects of local anesthetics and drugs with local anesthetic-like properties, such as propranolol and mepacrine, are manifested on Ca^{2+}-dependent processes [25] and have usually been attributed to influences on membrane Ca^{2+} permeability or binding [26]. Ca^{2+} binds tightly to polyphosphoinositides [27]. The finding that propranolol and mepacrine enhance poly PI biosynthesis by activating the enzymes involved in their biosynthesis may suggest that these drugs could exert their local anesthetic-like properties by increasing the synthesis of these acidic phospholipids and subsequently the binding of Ca^{2+} to the plasma membrane of the cell.

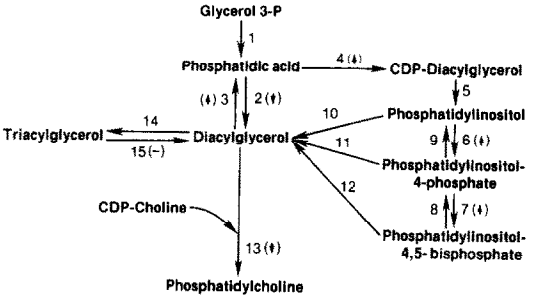

Fig. 3. Summary of the effects of mepacrine and propranolol on the enzymes involved in glycerolipid synthesis: Key (↑) Stimulation; (↓) inhibition; and (—) no effect. The enzyme activities referred to in the figure and text are indicated as follows: (a) Sn-glycerol-3-phosphate acyltransferase; (2) PA phosphohydrolase; (3) DG kinase; (4) CDP-DG synthetase; (5) PI synthase; (6) PI kinase; (7) PI-4-P kinase; (8) PI-4,5-bisphosphate phosphomonoesterase; (9) PI-4-phosphate phosphomonoesterase; (10) PI phosphodiesterase; (11) PI-4-P phosphodiesterase; (12) PI-4,5-bisphosphate phosphodiesterase; (13) DG cholinephosphotransferase; (14) DG acyltransferase; and (15) TG lipase.

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