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INCREASE OF PHOSPHATIDYLINOSITOL ARACHIDONIC ACID INCORPORATION INDUCED BY MEPACRINE

NORMA B. STERIN-SPEZIALE,* CLARA P. SETTON, VERÓNICA L. KAHANE and EMIR H. SPEZIALE

Department of Biological Chemistry, Buenos Aires University, Buenos Aires, Argentina

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Abstract—In view of the fact that mepacrine (Mp) is usually used as an inhibitor of the endogenous phospholipase A_2, and since this enzyme produces the release of arachidonic acid (AA) from membrane phospholipids, we studied the effect of different concentrations of Mp on the mobilization of [1-^{14}C]AA in rat renomedullary phospholipids. During the acylation period, 0.1 mM Mp did not produce any significant change in the incorporation of [1-^{14}C]AA into phosphatidylcholine (PC) and phosphatidylethanolamine (PE), and only a slight increase in phosphatidylinositol (PI). Higher concentrations of Mp (0.5 to 1.0 mM) produced a decrease of radioactivity in PE and PC with an increase in PI. Using prelabeled slices, a dose-dependent decrease in the ^{14}C-radioactivity in PE and PC was observed, with a parallel increase in PI. This effect of Mp persisted even in the presence of a physiological activator of phospholipase A_2, bradykinin (BK). No change in the net amount of phospholipids was observed at any of the Mp concentrations used. The results of this study show that Mp, at concentrations generally used to inhibit phospholipase A_2, produced a transfer of arachidonic acid from PE and PC to PI, rather than a blockade in the release of AA from membrane phospholipids.

It has been reported that, under circumstances where prostaglandin synthesis is stimulated, the release of arachidonic acid (AA) is remarkably specific compared with that of other fatty acids. The source of this specificity has been assumed to be a phospholipase A_2, specific for the release of arachidonyl moieties [1]. The synthesis of prostanoids is limited by the availability of free AA, of which the cytoplasmic level is controlled both by the liberation from the 2-position of esterified phospholipids and the resterification of the fatty acid into cellular phospholipid [2].

Arachidonic acid is not uniformly distributed among cellular phospholipids. Its specific lipid class distribution is maintained through continuous remodeling of phospholipid fatty acids by the deacylation–reacylation pathway initiated by the phospholipase A_2 activity. To demonstrate endogenous phospholipase A_2 activation is rather difficult, because the free fatty acid can be reutilized by reacylation. Many recent studies have utilized phospholipase A_2 inhibitors in an attempt to demonstrate the importance of phospholipase A_2 activation in the initiation of cellular responses. The agent that has been employed extensively is mepacrine (Mp), which has been shown to block the release of AA, a measure of phospholipase A_2 activity in several systems [3–5].

In the present study we report the influence of Mp on the distribution of AA among the rat renal medullary phospholipids during the acylating process, and the ability of the drug to induce redistribution of the fatty acid into phosphatidylinositol (PI) from phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

METHODS

Materials. [1-^{14}C]Arachidonic acid (55.0 μCi/mmol) was obtained from New England Nuclear. Mepacrine, bradykinin (BK) and the phospholipid standards were from Sigma. Kieselgel 60 F plates were from Merck A.C., and X-ray film for autoradiography was from the Eastman Kodak Co.

Solutions of mepacrine and bradykinin were prepared in Krebs medium.

Preparation of tissue slices. After decapitation of Wistar rats (250 g), both kidneys were removed and maintained in ice-cold Krebs solution. Each kidney was cut in half through the pelvis along its longitudinal axis, and the renal cortex and medulla were separated by scissors and scalpel dissection. The renal medullary tissue was sliced (approximately 0.5 mm thick), using a Stadie–Riggs microtome. For each experiment, 30 mg of tissue slices was collected in 0.5 ml of cold Krebs–Ringer bicarbonate buffer containing 5.5 mM glucose. To measure the effect of Mp on basal rates of acylation, the tissue was incubated at 37° with specific concentrations of Mp for 10 min. Then, 0.25 μCi of [1-^{14}C]AA was added and incubation was carried out for another 60 min under an atmosphere of 95% O_2–5% CO_2 in a metabolic shaking bath. The slices were washed and centrifuged three times with ice-cold Krebs solution, and resuspended in Krebs solution.

To study the effect of Mp on the redistribution of AA, the prelabeled slices were resuspended after three washings, three times with Krebs–albumin 1% (to eliminate the excess [1-^{14}C]AA), and reincubated for 30 min in the absence or presence of specific

* Correspondence: Dr. Norma Sterin-Speziale, Universidad de Buenos Aires, Facultad de Farmacia y Bioquimica, 1113 Junín 956, Buenos Aires, Argentina.

Table 1. Distribution of rat renal medullary phospholipids: Effect of mepacrine

Phospholipids	Phosphorus [ng/mg tissue (wet wt)]			
		Mepacrine (mM)		
	Control	0.1	0.5	1.0
Phosphatidylcholine	98.0 ± 5	94.0 ± 6	101.0 ± 7	105.0 ± 6
Phosphatidylinositol	25.5 ± 3	25.0 ± 2	27.0 ± 3	26.0 ± 3
Phosphatidylserine	41.2 ± 4	40.0 ± 3	41.0 ± 3	39.0 ± 4
Phosphatidylethanolamine	100.5 ± 9	104.5 ± 6	110.0 ± 9	103.0 ± 8

concentrations of Mp. The BK activation was achieved by incubation of the prelabled slices for 30 min in the presence of the hormone with or without the addition of different concentrations of Mp.

Lipid extraction and analysis. Incubations were stopped by adding 2.0 ml of chloroform:methanol (1:2, v/v) [6], and samples were homogenized in glass tubes with a Teflon pestle at 3000–3500 rpm. Phases were split by adding 0.6 ml of chloroform and 0.6 ml of water. The lower chloroform phase was removed and dried under a flow of nitrogen.

The residues were dissolved in 25 μl of chloroform and quantitatively applied to silica gel G plates. The plates were developed with chloroform:methanol:acetic acid:water (40:10:10:1, by vol.), dried and then redeveloped in the same direction to a level 1.2 cm below the first solvent, in chloroform:methanol:acetic acid:water (120:46:19:3 by vol.) [7]. The zones corresponding to the different standards were visualized by exposing the chromatograms to iodine vapors, and the radioactive zones were detected by autoradiography. Specific areas were scraped off, and the ^{14}C-radioactivity was quantitated in a liquid scintillation counter. The results are expressed as cpm/30 mg of tissue, and represent the mean ± SE of five experiments (Student's *t*-test).

For the quantitation of endogenous phospholipids, the appropriate areas of the thin-layer plates (TLC) were digested with 70% perchloric acid, and the resulting inorganic phosphate was assayed [8].

RESULTS

Distribution of phospholipids in rat renal medullary tissue. The different classes of phospholipids found in unlabeled rat renal medulla are presented in Table 1. The major phospholipids identified were PC and PE, which constituted 37 and 38% of the total phospholipids. Phosphatidylinositol and PS each contributed 9.5 and 15.5% respectively.

Incubation in the presence of 0.1 to 1 mM Mp did not modify this pattern of distribution, and the net amount of the total phospholipids remained constant.

No lysophospholipids were detected.

Effect of mepacrine on arachidonic acid incorporation into phosphatidylinositol. Figure 1 shows that Mp produced a concentration-dependent increase in radioactive arachidonic acid incorporation into rat renal medullary PI during the acylation process. The highest stimulation attained by PI was 280% of the value observed in the absence of the drug. On the contrary, 1.0 mM Mp caused a 37 and 54% decrease of radioactivity in PC and PE respectively.

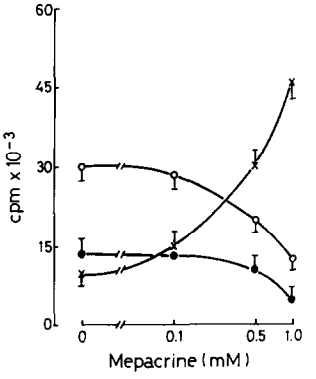

Fig. 1. Effect of MP on AA incorporation in rat renomedullary phospholipids. Key: (○) phosphatidylcholine, (●) phosphatidylethanolamine, and (×) phosphatidylinositol. Each point is the mean (±SE) of five observations.

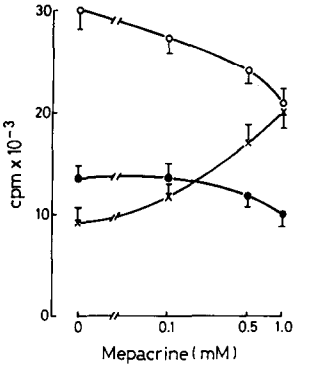

Fig. 2. Redistribution of AA among the phospholipids, induced by Mp. Key: (○) phosphatidylcholine, (●) phosphatidylethanolamine, and (×) phosphatidylinositol. Each point is the mean (±SE) of five observations.

When prelabeled rat renal medullary slices were reincubated in fresh buffer in the presence of Mp (Fig. 2), a dose-dependent increase in the PI arachidonic acid incorporation was observed. This increase

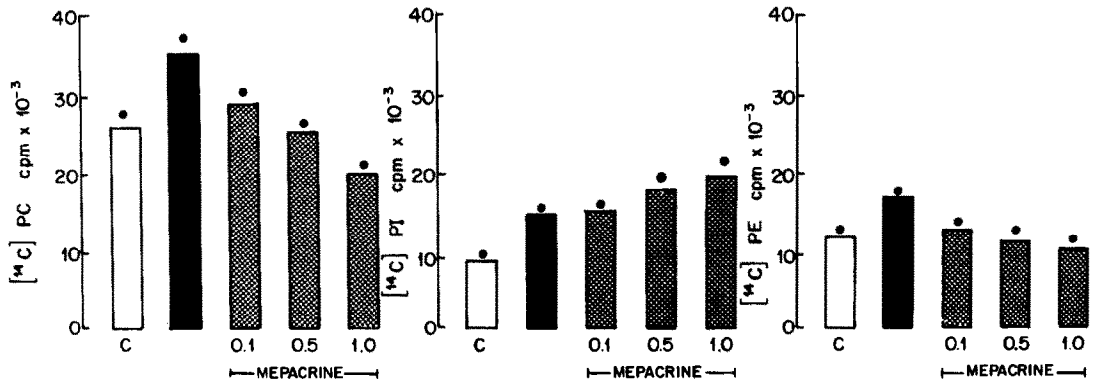

Fig. 3. Effect of Mp on AA redistribution of prelabeled slices activated with BK. Key: (□) control, (■) BK (1 mg/ml), and (▩) BK + Mp. Each bar is the mean (±SE) of six observations.

was accompanied, at any concentration used, by a decrease in the labeling of PC and PE. The maximal incorporation obtained by PI was 115% of the control value, with a concomitant decrease in PC and PE of 24 and 19%. Under these conditions the amount of radioactivity gained by PI corresponded to that lost by PC and PE. It is interesting to note that, during the acylation period, when an excess of [1-^{14}C]AA was present, an increase in the total radioactivity incorporated in the phospholipids of 10^4 cpm was obtained. At the lower concentration of Mp, the radioactivity gained was accumulated into PI, with no significant changes in the other phospholipids. At the higher concentration of Mp, the increase in PI was accompanied by a decrease in phosphatidylcholine and phosphatidylethanolamine. Thus, at 0.5 mM Mp, the PI increase was 2.3×10^4 cpm and the decrease in PC and PE together was 1.3×10^4 cpm. At the highest concentration of Mp, PI gained 3.6×10^4 cpm and the loss in PC and PE radioactivity was 2.5×10^4 cpm. In the experiments where we used prelabeled slices, equilibrium was reached and no excess of arachidonic acid was present, and the total amount of radioactivity incorporated did not change. The distribution among the different phospholipids was affected by the different concentrations of Mp. Thus, at 0.1 mM mepacrine, PI gained 2.3×10^3 cpm that were lost by PC. The higher concentration of mepacrine made the phenomenon more significant and induced a decrease in the PE radioactivity. Phosphatidylserine did not incorporate radioactive arachidonic acid, and no changes were observed in the presence of Mp. Triglycerides (TG) incorporated a considerable amount of radioactive AA, but Mp, at any of the concentrations used, did not induce its mobilization (data not shown).

Figure 3 summarizes the results obtained under the physiological stimulation of the prelabeled slices. After 30 min of incubation, BK induced an increase of the radioactive AA incorporation into PC, PI and PE of 33, 70 and 46% respectively. The increase was produced as a consequence of the activation of the deacylation–reacylation cycle, and the source of the [^{14}C]AA seems to be that stored in TG.*

When, in addition to BK, different concentrations of Mp were added to the incubation medium (Fig. 3), a concentration-dependent decrease in the PC and PE radioactivity was observed with an increase in PI. The lower concentration of Mp used (0.1 mM) produced a decrease in PC and PE of 19.5 and 26%, respectively, with no changes in PI. At a 0.5 mM concentration, Mp completely reversed the increase produced by BK on the radioactive AA incorporated into PC and PE. In contrast, a 23% increase in PI radioactivity was observed with respect to that produced by BK. At the highest concentration of Mp (1 mM), the values in PC and PE radioactivity were even lower than those obtained in the absence of BK, while the radioactivity in PI was 32% more than the value obtained in the presence of BK alone.

Under these experimental conditions the loss of radioactivity in PC and PE, produced by Mp, was not totally regained by PI. The maximum incorporation into PI gave values similar to those obtained in the absence of BK (Fig. 2).

DISCUSSION

The results of this study indicate that mepacrine, an anti-malarial drug which has been utilized as a phospholipase A_2 inhibitor in many systems [1–4], induces redistribution of AA, possibly by a transacylation phenomenon. The concentrations of mepacrine required to produce this effect were in the range in which it is usually used as a phospholipase A_2 inhibitor.

The fact that, during the acylation period, the radioactivity incorporated into phosphatidylinositol in the presence of Mp was higher than that lost by PC and PE (Fig. 1) indicates that the source of the arachidonic acid could be the excess in the incubation medium. In contrast, using prelabeled slices (Fig. 2), it appears that a real transfer of AA to PI occurred. Most of the authors have used Mp as a phospholipase A_2 inhibitor on the basis of its capacity to block the release of AA and, consequently, to diminish prostaglandin synthesis [1–3, 8, 9]. But the direct effect of Mp on AA mobilization has not been studied extensively. The present study demonstrates that, rather than inhibiting the release of arachidonic acid, mepacrine produced a redistribution of this fatty

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acid. This effect of Mp was observed even in the presence of a physiological activator of phospholipase A_2, such as BK (Fig. 3). This kind of stabilization of AA in PI may diminish the availability of the precursor for the prostaglandin synthesis. On the other hand, since PC has been considered to be the main source of AA under conditions of phospholipase A_2 activation [10, 11], this kind of depletion in the fatty acid reported here could reduce the storage of the substrate for the phospholipase A_2 activity.

Another consequence of the redistribution of AA could be a change in the physical state of the substrate that characterizes the phospholipase A_2 specificity [12, 13], and it could explain the resistance of different cell types to specific stimuli in the presence of Mp. The mechanism by which mepacrine induces arachidonic acid redistribution is not clear, but it could occur through the pathway described by Irvine and Dawson [1]. Two mechanisms may be involved in arachidonic acid incorporation into phospholipids: the Lands pathway [14] and the CoA-mediated arachidonic acid transfer, first described by Irvine and Dawson in rat liver microsomes. Lands pathway can account for the synthesis of arachidonoyl phospholipids.

The CoA-mediated transfer is suggested by the arachidonyl transfer from PC to PI. This CoA-mediated transfer is independent of phospholipase A_2 and is the preferred pathway for the AA distribution of phospholipids in conditions of low levels of ATP [15]. The reason we suggest this route as an explanation for the Mp arachidonic acid redistribution is that, among the various effects of Mp, inhibition of oxidative phosphorylation [16] and glucose oxidation [17, 18] has been described in several systems. This mepacrine metabolic effect may decrease the amount of ATP in the system and favor the CoA-mediated route for AA redistribution. Other studies reported the formation of a complex between mepacrine and the phospholipids, and the authors suggest that the drug–phospholipid complex may be resistant to degradation by phospholipases [13, 19]. In our experimental conditions we cannot assess the formation of a Mp–phospholipid complex, but the excess of Mp extracted with the phospholipids comigrated with PS in the TLC plate. Since PS did not incorporate AA in our system, the presence of mepacrine does not change the interpretation of the results. On the other hand, we were unable to demonstrate the presence of any new iodine or radioactive spot in the TLC plate as has been described by Dise *et al.* [20].

We conclude that mepacrine, at concentrations used to inhibit phospholipase A_2, produced in rat renal medulla a rearrangement of AA to the PI pool without affecting the net amount of the phospholipids, and we suggest that this redistribution of the fatty acid could result in a resistance to phospholipase A_2 degradation.

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