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Regulation of Phospholipid Degradation and Biosynthesis in the Heart by Isoprenaline: Effect of Mepacrine

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Summary

We investigated the effect of isoprenaline (IPRO), a β-mimetic catecholamine, on incorporation of (^{32}P)Pi into phospholipids of the mouse left ventricle *in vivo*. All experimental groups of male mice received an injection of (^{32}P)Pi (250 MBq x kg^{-1} b.w.) intraperitoneally two hours prior to sacrifice. A single dose of IPRO (5 mg x kg^{-1} b.w.) was injected one hour before killing. IPRO increased the specific radioactivity of phosphatidylcholine (PC) by a factor of 1.8, diphosphatidylglycerol (DPG) 2.1, sphingomyelin (SM) 3.5, phosphatidylinositol (PI) 1.7, phosphatidylserine (PS) 1.7, phosphatidylglycerol (PG) 1.7, phosphatidic acid (PA) 2.0 compared to control values. On the other hand, IPRO is also known to stimulate phospholipid degradation by activation of phospholipase A_2. That is why we used mepacrine (50 mg x kg^{-1} b.w.), a phospholipase inhibitor, to find a possible link between biosynthesis and degradation of phospholipids. Pretreatment with mepacrine two hours prior to sacrifice suppressed IPRO stimulated incorporation of (^{32}P)Pi into phospholipids nearly to control levels. Mepacrine itself did not significantly influence the specific radioactivity of phospholipids. We conclude that phospholipase A_2 inhibitor, mepacrine, is able to prevent IPRO-stimulated icorporation into phospholipids, suggesting a feedback relation between their biosynthesis and degradation in the myocardium.

Key words

Phospholipids – Mepacrine – Isoprenaline – Heart

Introduction

Membrane phospholipids are essential factors in maintaining cell integrity and function. Their accelerated degradation is supposed to be one of the critical events in infarct-like heart injury produced by β-mimetic catecholamine isoprenaline (IPRO) (Kondo *et al.* 1987). A high single dose of IPRO decreased the phospholipid content in the rat heart (Okumura *et al.* 1983, Chatelain *et al.* 1987). A similar observation was reported for the carp and mouse heart after repeated administration of the drug (Drnková *et al.* 1988, 1990). This effect has been explained by phospholipase A_2 activation (Franson *et al.* 1979, Kondo *et al.* 1987) and can be prevented by phospholipase A_2 inhibitors (Otamiri *et al.* 1988, Takasu *et al.* 1989).

On the other hand, Vorbeck *et al.* (1975) found an increased incorporation of radioactive phosphate into phospholipids of the rat heart shortly after *in vivo* administration of IPRO. We observed both a decreased content of phospholipids and a subsequent increase in their rate of biosynthesis in the denervated insect flight muscle (Nováková *et al.* 1988).

The purpose of the present paper was to elucidate whether the phospholipase A_2 inhibitor mepacrine (Chang *et al.* 1987) is able to prevent IPRO-stimulated incorporation of radioactive phosphate into phospholipids, suggesting a possible feedback relation with their degradation.

Material and Methods

Male mice SPF-ICR (30–35 g, b.w.) Velaz were used throughout this study. They were kept under standard conditions and fed *ad libitum*. IPRO (Spofa) 5 mg x kg^{-1}, mepacrine (Sigma) 50 mg x kg^{-1}, (^{32}P)Pi

250 MBq x kg^{-1} body weight were dissolved just before application in 200 μl of physiological saline to protect expecially IPRO against oxidation. The control mice received an injection of (^{32}P)Pi in physiological saline. All solutions were injected intraperitoneally.

The experiments with IPRO and mepacrine were carried out on four groups of mice (I–IV). All mice were injected (^{32}P)Pi two hours before sacrifice. Mepacrine was given to groups II and IV concomitantly with (^{32}P)Pi. Group I (control) and group II (mepacrine) received physiological saline one hour after the radioactive phosphate injection, at the same time group III (IPRO) and group IV (mepacrine+IPRO) received IPRO.

The left ventricle of the heart was excised after killing the animal. The tissue was frozen in liquid nitrogen and pulverized. Phospholipids were extracted according to a modified method of Folch *et al.* (1959) in three subsequent portions (3 ml each) of a mixture of chloroform and methanol (1:3, 2:1 and 2:1).

The extract was evaporated under nitrogen and phospholipids were separated by two-dimensional thin-layer chromatography. Silica Gel H (Merck) as a slurry of 22.5 g in 62 ml of water containing 2.5 g of Magnon (Merck) was spread with a 0.25 mm fixed spreader (Desaga) on glass plates (20 x 20 cm). The plates were developed in the first dimension with chloroform-methanol-water (65:25:4) and in the second with 1-butanol-acetic acid-water (60:20:20) according to a modified method of Rouser *et al.* (1969). This method resulted in adequate separation of PC, PE, DPG, SM, PG, LPE, LPC. For a good separation of PA, PI and PS after 2 h of (^{32}P)Pi incorporation (used throughout the experiments with IPRO and mepacrine) the method of Rouser *et al.* (1970) was used. The phospholipid spots were visualized by iodine vapours, scraped out and analysed for phosphorus (Rouser *et al.* 1970).

The radioactivity of parallel spots was measured by a liquid scintilation counter Nuclear Chicago. The specific radioactivity of (^{32}P)phosphocreatine was determined in the whole heart (Kopp and Bárány 1979).

Student's t-test was used for statistical evaluation.

Table 1
The time course of (^{32}P)Pi incorporation in phosphocreatine and phospholipids

	Specific radioactivity (cpm x μmol P^{-1})			
Control	1 hour	2 hours	3 hours	4 hours
PCr	45080 ± 19520	207326 ± 18066	311411 ± 18425	225544 ± 13360
PC	3055 ± 731	8439 ± 2762	17434 ± 1943	22453 ± 3081
PE	745 ± 101	2893 ± 1105	4335 ± 410	5765 ± 280
PI+PS	6593 ± 398	13056 ± 986	16467 ± 786	21151 ± 3584
PG	3055 ± 532	9671 ± 2362	12678 ± 2842	15751 ± 652
SM	144 ± 35	708 ± 227	1950 ± 301	3043 ± 1050
DPG	51 ± 13	281 ± 92	524 ± 100	1017 ± 146
PA		45536 ± 12606		
PI		18034 ± 448		
PS		2280 ± 608		

(^{32}P)Pi was injected i.p. 2 MBq in 0.2 ml of physiological saline. Each value represents the average of five determinations ± S.E.M. PCr (phosphocreatine), PC (phosphatidylcholine), PE (phosphatidylethanolamine), PI (phosphatidylinositol), PG (phosphatidylglycerol), SM (sphingomyelin), DPG (diphosphatidylglycerol), PA (phosphatidic acid), PS (phosphatidylserine), P (phospholipid phosphorus)

Results

The time course of (^{32}P)Pi incorporation in phosphocreatine and phospholipids

The specific radioactivities of phosphocreatine (PCr) and phospholipids were estimated in the control animals after i.p. injection of (^{32}P)Pi one, two, three and four hours prior to sacrifice.

Table 1 shows that the incorporation curve of PCr is bell-shaped with maximum appearing at three hours after the (^{32}P)Pi injection. The specific

radioactivity of all phospholipids continually rises during the four-hour interval followed. The labelling rates of individual phospholipids differ. Their magnitudes decline in the order: PI+PS, phosphatidylcholine (PC), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), sphingomyelin (SM) and diphosphatidylglycerol (DPG). The results in Table 1 indicate that the time suitable for radionuclide administration is two hours prior to sacrifice. For this time interval the specific radioactivities of phosphatidic acid (PA), phosphatidylinositol (PI) and phosphatidylserine (PS) are also presented. The highest specific radioactivity was found in PA.

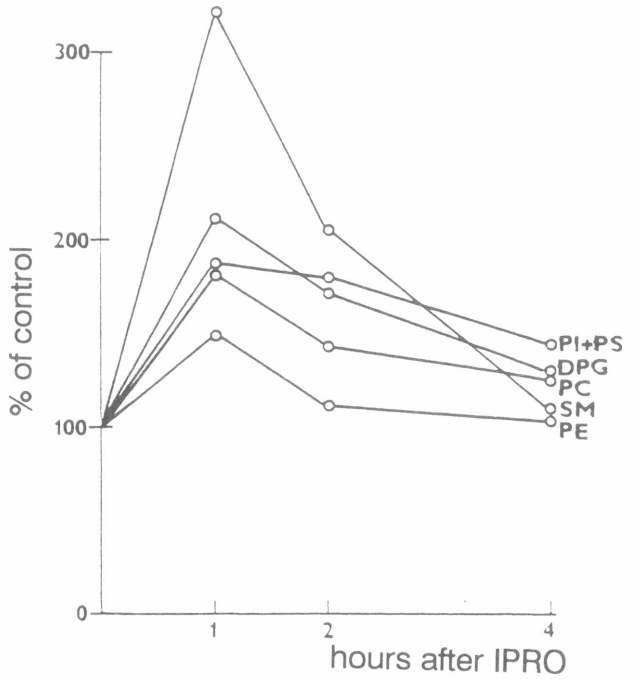

Fig. 1
Incorporation of (^{32}P)Pi (250 MBq x kg^{-1} b.w.) after isoprenaline (IPRO 5 mg x kg^{-1} b. w.) administration. (^{32}P)Pi was injected two hours and IPRO one, two and four hours prior to sacrifice. Each point represents the mean of three experiments.
PC (phosphatidylcholine),
PE (phosphatidylethanolamine),
PS (phosphatidylserine),
SM (sphingomyelin),
DPG (diphosphatidylglycerol),
PI (phosphatidylinositol).

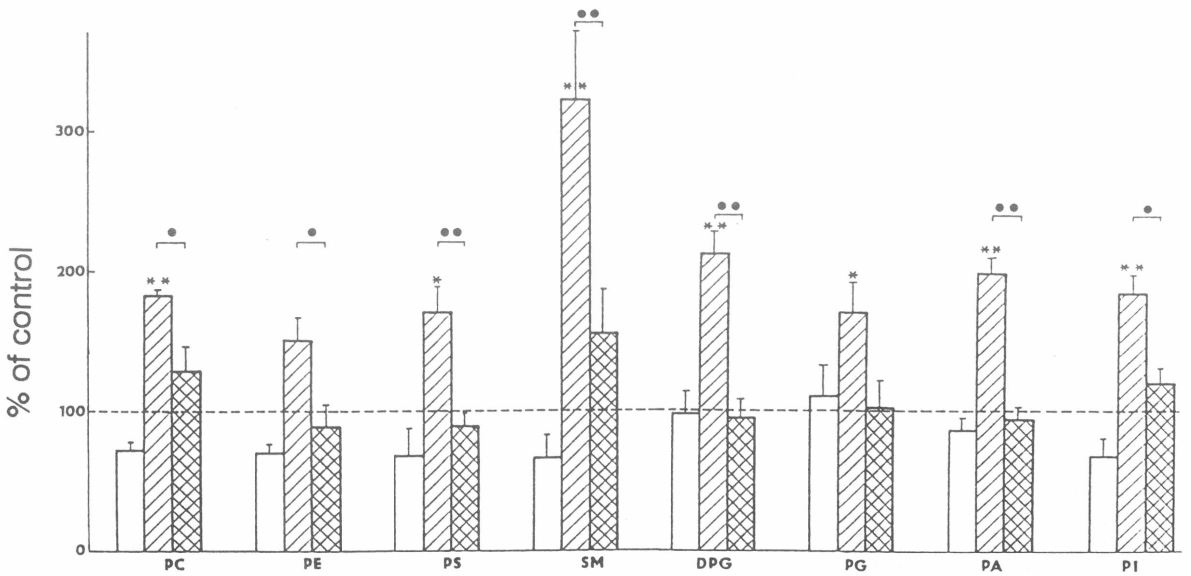

Fig. 2
Specific radioactivity of phospholipids in the left ventricle expressed as percentage of the control (100 % broken line). (^{32}P)Pi (250 MBq x kg^{-1} b. w.) and mepacrine (50 mg x kg^{-1} b. w.) was injected two hours and isoprenaline (IPRO 5 mg x kg^{-1} b. w.) one hour before sacrifice. Open squares – mepacrine, hatched squares – IPRO, cross-hatched squares – mepacrine + IPRO. Statistical significance: * $p<0.05$ and ** $p<0.01$ IPRO versus single black dot $p<0.05$, two black dots $p<0.01$ mepacrine+IPRO versus IPRO. Each column represents the mean of six experiments. Vertical bars represent S.E.M.

The specific radioactivity of phospholipids after IPRO treatment

Specific radioactivities of phospholipids were measured one, two and four hours after IPRO administration. Radioactive phosphate was given to each experimental group two hours prior to sacrifice. Fig. 1 shows that IPRO-stimulated incorporation of radioactive phosphate into phospholipids was maximal one hour after administration of the radionuclide. The most affected phospholipids were SM and DPG. The specific radioactivities returned almost to control levels four hours after IPRO treatment.

One hour after drug injection, no difference in labelling of the precursor pool could be detected in IPRO-treated animals. The specific radioactivity of (^{32}P)PCr in controls was 207326±18066 and 181169±22378 cpm x μmol P^{-1} after IPRO administration.

The influence of IPRO and mepacrine on (^{32}P)Pi labelling and the content of phospholipids

The experiments presented in Fig. 2 were carried out on four groups of mice (I–IV) (see Methods). Mepacrine itself did not significantly influence (^{32}P)Pi incorporation into any of the phospholipids. On the other hand, IPRO considerably enhanced the specific radioactivities of phospholipids (PC 1.8, PE 1.5 n.s., PS 1.7, SM 3.5, DPG 2.1, PG 1.7, PA 2.0 and PI 1.7 times in comparison with the controls). Mepacrine suppressed IPRO-stimulated incorporation almost to control values.

No changes in the net amount of phospholipids were observed in any of the experimental groups (Table 2).

Table 2
The concentration of phospholipids in the left ventricle of the mouse heart

	μmol P x g^{-1} wet weight			
	I Control	II Mepacrine	III IPRO	IV IPRO+Mepacrine
PC	14.69±0.30	14.77±0.42	14.67±0.45	14.53±0.31
PE	10.53±0.12	10.90±0.44	10.86±0.42	10.24±0.51
PS	0.78±0.05	0.83±0.03	0.83±0.05	0.72±0.03
SM	0.81±0.17	0.87±0.09	0.83±0.05	0.81±0.09
DPG	4.79±0.31	4.62±0.25	4.26±0.18	4.28±0.23
PG	0.42±0.08	0.40±0.03	0.47±0.09	0.50±0.06
PA	0.26±0.01	0.22±0.04	0.20±0.03	0.22±0.02
PI	1.33±0.02	1.38±0.12	1.42±0.04	1.40±0.07
LPE	0.05±0.03	0.06±0.01	0.06±0.01	0.06±0.02
LPC	0.27±0.04	0.20±0.02	0.24±0.08	0.25±0.09

Mepacrine (50 mg x kg^{-1}) was injected two hours and isoprenaline (IPRO 5 mg x kg^{-1}) one hour before sacrifice. Each value represents the mean of seven determinations ± S.E.M.. PC (phosphatidylcholine), PE (phosphatidylethanolamine), PS (phosphatidylserine), SM (sphingomyelin), DPG (diphosphatidylglycerol), PG (phosphatidylglycerol), PA (phosphatidic acid), PI (phosphatidylinositol), LPE (lysophosphatidylethanolamine), LPC (lysophosphatidylcholine), P (phospholipid phosphorus)

Discussion

The direct precursor for phospholipid phosphorus is the γ-phosphate of ATP which equilibrates with phosphate of PCr in the heart very rapidly (Ericson-Viitanen *et al.* 1982). The incorporation time of (^{32}P)Pi was two hours in all our experiments. The specific radioactivities of both PCr and phospholipids in control hearts were still in ascending phase of their incorporation curves. The highest specific radioactivity of phospholipids (in phosphatidic acid) was four times lower than the specific radioactivity of PCr. Thus the radioactivity of phospholipids did not attain equilibrium with the specific radioactivity of the precursor and there was sufficient space for the eventual increase in their specific radioactivity caused by IPRO treatment.

We observed that (^{32}P)Pi incorporation was increased into all phospholipid species in the mouse left ventricle which reached a maximum 60 min after IPRO administration. The highest effect was measured in the case of SM and DPG. Since the plasma membrane is the major location for SM, whereas DPG is localized almost exclusively in the mitochondria, our results suggest that both types of membranes are involved in the IPRO effect.

Vorbeck *et al.* (1975) reported that phospholipid labelling and the specific activity of CTP: PA cytidylyltransferase are enhanced in the IPRO-treated rat heart. This enzyme is involved in *de novo* synthesis of PI, PG and DPG. Jacab *et al.* (1988) found stimulation of phospholipid biosynthesis in the sarcoplasmic reticulum of isolated guinea-pig hearts influenced by IPRO. We have also demonstrated an increase (approximately by 100 %) in the specific radioactivity of PA. The specific activity of 1-acyl-sn-glycero-3-phosphate acyltransferase, the enzyme participating in PA *de novo* synthesis, was increased in rat parotid salivary glands after IPRO treatment (Yashiro *et al.* 1988). All these experimental data are consistent with the stimulation of phospholipid *de novo* synthesis in the IPRO-treated myocardium which we have observed.

On the other hand, it has been well documented that the high dose of IPRO (40 mg x kg^{-1}) has a stimulatory effect on phospholipid degradation in the rat heart. The concentration of phospholipids was decreased and their degradation products, lysophospholipids and free fatty acids, were increased 24 hours after IPRO administration. The pretreatment of IPRO-treated rats with Ca^{2+} channel blockers and phospholipase inhibitors prevented myocardial damage and phospholipid degradation (Okumura *et al.* 1983, Chatelein *et al.* 1987, Takasu *et al.* 1989). These results demonstrated the important role of Ca^{2+}-dependent phospholipase activation in the development of myocardial injury by IPRO.

An increase in phospholipase activity in the heart homogenates from IPRO-treated rats was found by Kondo *et al.* (1987). Stimulation of phospholipase A_2 activity in isolated canine cardiac sarcolemma by IPRO *in vitro* was also reported (Franson *et al.* 1979).

Studies dealing with the regulation of PC biosynthesis have provided evidence that the decrease in concentration of PC and/or the increase in free fatty acid levels stimulated the activity of the regulatory enzyme of the PC biosynthetic pathway CTP:phosphocholine cytidylyltransferase by translocation of the enzyme from the cytosolic fraction to the more active microsomal fraction of hepatocytes (Vance 1990). The same effect was found with phospholipase A_2, phospholipase A_2 activator mellitin and Ca^{2+} (Aeberhard *et al.* 1986, Sanghera and Vance 1989, 1990). Hypoxic treatment resulted in the enhanced translocation of cytidylyltransferase from cytosolic to microsomal form in the hamster heart caused by accumulation of fatty acids during hypoxia (Hatch and Choy 1990).

Under our experimental conditions, we did not observe any change in the concentration of phospholipids and their degradation products, namely the lysophospholipids. The possible explanation is that the rates of degradation and synthesis of phospholipids are highly coordinated during the 60 min period of IPRO (5 mg x kg^{-1}) treatment. Therefore, a small decrease in the content of phospholipids could not be detected.

Nevertheless, we have shown that mepacrine, an inhibitor of phospholipase A_2, suppressed the increased incorporation of radioactive phosphate after IPRO treatment. We assumed that mepacrine inhibited phospholipase A_2 activity and thus prevented phospholipid degradation. This may be the reason why IPRO-stimulated biosynthesis was also suppressed. Our results have brought further evidence for a direct link between degradation and synthesis of phospholipids. It suggests that IPRO-stimulated biosynthesis of phospholipids is a compensatory process maintaining a steady level of phospholipids in cell membranes thus presenting new aspects of feedback relations between phospholipid breakdown and biosynthesis. Such a homeostatic mechanism would stabilise the level of phospholipids in the cell despite fluctuations in their degradation rate.

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Reprint Requests

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