THE EFFECT OF SOME ANTIBIOTICS ON ENDOGENOUS PHOSPHOLIPASE ACTIVITY IN RAT LIVER

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(Received for publication February 25, 1984)

In relation to a previous paper (J. Antibiotics 32: 734~739, 1979), the effect of some antibiotics on endogenous phospholipid degradation of rat liver was investigated by slice and perfusion techniques. Polymyxin B inhibited this degradation most strongly, benzylpenicillin, chloramphenicol and peplomycin inhibited moderately, and carbenicillin, cephaloridine and streptomycin did not inhibit. Tetracaine and ethylenediaminetetraacetic acid, which are not antibiotics, also showed inhibitory effects.

The results strongly suggest that besides their usual antibiotic actions, some antibiotics may participate in endogenous phospholipid metabolism and biomembrane functions of host cells.

Under hypoxic conditions, cells of various organs, such as rat liver[1], and kidney[2], are injured and die, possibly owing to the endogenous phospholipase activity, phospholipid degradation and dysfunction of biomembranes.

In previous papers[3,4], the effect of some antibiotics on phospholipase activity *in vitro* was reported. In this paper, to more closely simulate an *in vivo* system, slice and perfusion techniques were employed in which the effect of 7 antibiotics and 2 other compounds on endogenous phospholipase activity of rat liver was examined. Some of them were found to inhibit endogenous phospholipid degradation.

The results strongly suggest that some antibiotics participate in endogenous phospholipid metabolism and biomembrane function of host cells in addition to their usual antibiotic actions.

Materials and Methods

The antibiotics tested were as follows: benzylpenicillin potassium, streptomycin sulfate (Meiji), carbenicillin disodium, polymyxin B sulfate (Pfizer-Taito), cephaloridine (Shionogi), chloramphenicol sodium succinate (Sankyo), peplomycin (pepleomycin) sulfate (Nippon Kayaku). Tetracaine hydrochloride (Kyorin) and ethylenediaminetetraacetic acid (EDTA) disodium were also used. The heat-treated phospholipase A_2[5] was free from protease, phosphodiesterase, AMPase activities and had a specific activity of 38.5 for egg yolk phosphatidylcholine. Melittin, No. M-7129 from bee venom, was obtained from Sigma. This is a membrane-active peptide and stimulates the activity of an endogenous as well as an added phospholipase A_2. Its maximal enzymatic hydrolysis is observed at melittin-phospholipid ratios of about 1: 100[6]. [1-^{14}C]Arachidonic acid (55.8 mCi/mmol) was a New England Nuclear product.

Preparation of Rat Liver Slice Labelled and Non-labelled with [1-^{14}C]Arachidonic Acid

Principally, by the method of ÅKESSON *et al.*[7], [1-^{14}C]arachidonic acid, 9.45×10^5 dpm, was dissolved in 1 ml hexane and stirred with 5 ml of 10% bovine serum albumin solution in 0.1 N NaOH, pH 8.0 for 20 minutes in a Ultra Turrax (IKA Werke Co., F.R.G.). The hypolayer was aspirated, 0.7 ml of

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which, 5.94×10^5 dpm, was injected into a portal vein of male Wistar rat (about 200 g). The rat had been fasted for 24 hours and was anesthetized with pentobarbital (50 mg/kg). After 30 minutes, the liver was removed, washed with 0.9% saline and sliced with a slicer (Natsume, Japan). The diameter of the round slice was 26 mm and the wet weight about 100 mg, which contained about 150 μg lipid-phosphorus. Non-labelled slice was prepared similarly without the administration of [1-^{14}C]arachidonic acid. One slice was used for control and the others were for main experiments. When two or more rats were needed for an experiment, those who were similar in age and body weight were used.

Assay System for the Endogenous Phospholipid Degradation in Liver Slice

The slice was incubated in 2 ml Hanks buffer, pH 7.2, containing *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethylene sulfonic acid (HEPES), in a 50-ml glass stoppered tube for definite time intervals in the presence or absence of antibiotics. The slice was removed and the total lipids were extracted[8]. The separation of simple lipids and phospholipids was carried out by column chromatography on silicic acid[9], and each component of phospholipids was separated by preparative thin-layer chromatography in a solvent mixture of $CHCl_3$ - CH_3OH - H_2O (65: 35: 8), detected with Rhodamine 6G, scraped off the plate, extracted and the phosphorus was determined.

The endogenous phospholipase activity was expressed by percentage decrease in lipid-phosphorus content of the slice. The inhibitory action of antibiotics on the enzymatic reaction was expressed as percent inhibition of the decrease in lipid-phosphorus of the slice. ID_{50} is the concentration of antibiotics which cause 50% inhibition of the decrease in phospholipid content of the slice during a 4-hour incubation period.

Liver Perfusion with Heat-treated Phospholipase A_2, Melittin and Polymyxin B

Male Wistar rats (about 200 g) were fasted for 24 hours and anesthetized with pentobarbital and perfused with Hanks buffer, pH 7.4, containing 10 mM HEPES at a flow rate of 30 ml per 1 minute, according to the procedure of SEGLEN[10]. The perfusate contained heat-treated phospholipase A_2 (*Crotalus adamanteus*), melittin or antibiotics. After perfusion for definite time intervals, about 1 g liver tissue was removed from 5 different sites of the liver and phospholipids of each were analyzed.

Results

The Change in Phospholipid Content of Rat Liver Slice with Time

A typical degradation pattern of endogenous phospholipids in rat liver slice is shown in Fig. 1, and the distribution of radioactivity derived from [1-^{14}C]arachidonic acid into simple lipids and phospholipids is presented in Fig. 2. Since the decrease in content and radioactivity of phospholipids was similar, non-labelled slice was used in subsequent experiments.

Table 1. Inhibitory effect of some antibiotics on endogenous phospholipid degradation in rat liver slice.

Antibiotics	Remaining phospholipid content in liver slice (μg lipid-phosphorus/g liver slice)			
	Concentration of antibiotics (mM)			
	0	3	5	10
Benzylpenicillin, n=5		76.0±4.8	78.4±1.5	77.4±4.8
Carbenicillin, n=4		75.6±1.4	73.0±2.7	71.5±1.9
Cephaloridine, n=5		72.5±2.9	74.8±6.2	75.8±8.7
Chloramphenicol, n=6		77.4±2.4	79.0±8.7	82.7±4.7
Streptomycin, n=4		66.6±3.7	68.6±5.1	72.8±3.7
Polymyxin B, n=5		92.0±4.3	91.7±8.3	92.8±2.9
Peplomycin, n=3		74.3±2.5	78.5±1.9	77.1±3.7
Tetracaine, n=3		80.0±7.1	88.1±6.6	94.9±3.0
EDTA·2Na, n=3		81.7±1.6	89.0±6.1	89.4±1.9
Control, n=38	72.3±5.1			

Fig. 1. The endogenous phospholipid degradation in rat liver slice with time.

The solid line indicates lipid-phosphorus, the dotted line ^{14}C.

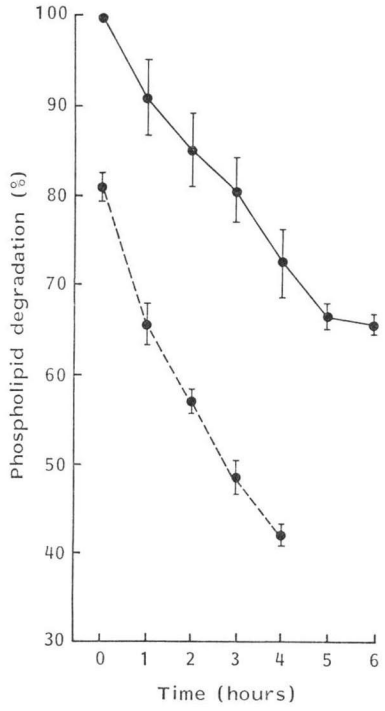
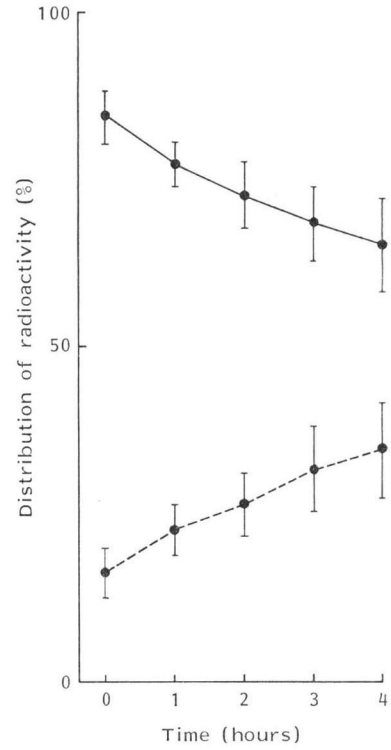


Fig. 2. The change in distribution of ^{14}C into simple lipids and phospholipids with time.

The radioactivity was derived from [1-^{14}C]-arachidonic acid. The solid line indicates phospholipids, the dotted line simple lipids.



When the glass tube was filled with a N_2 - CO_2 mixture (92: 8), instead of air, no difference in degradation of endogenous phospholipids was observed. The liver tissue, on a slice level, might be not so sensitive to distinguish between the air and a N_2 - CO_2 mixture.

The Effects of Antibiotics on Endogenous Phospholipid Degradation in Slice

Among the antibiotics tested, polymyxin B inhibited most strongly the endogenous phospholipid degradation, and benzylpenicillin, chloramphenicol and peplomycin inhibited moderately. Carbenicillin, cephaloridine and streptomycin, on the other hand, did not inhibit. Tetracaine, a local anesthetic, known as an inhibitor of phospholipase A_2 activity, showed a strong inhibitory effect on endogenous phospholipid degradation. EDTA inhibited the phospholipid degradation presumably owing to the chelation of Ca^{++}, which is an essential factor for phospholipase A_2 activity, but even at 10 mM concentration, the phospholipid degradation was not prevented completely (Table 1).

In case of benzylpenicillin, for example (Table 2), dose dependent inhibition was observed and similar results were also found in pancreas slice as reported elsewhere[11].

Effect of Polymyxin B on Melittin-induced Phospholipid Degradation at Liver Perfusion

Since some antibiotics, particularly polymyxin B, inhibited the endogenous phospholipid degrada-

Table 2. Dose-dependent inhibition of benzylpenicillin on endogenous phospholipid degradation in rat liver slice.

Concentration (mM)	Remaining phospholipids in liver slice after 4-hour incubation (μg lipid-phosphorus/g liver slice)
1	73.7±4.4
3	76.0±4.8
5	78.4±1.5
10	77.4±4.8
20	82.2±6.3
30	89.0±5.4

n=5

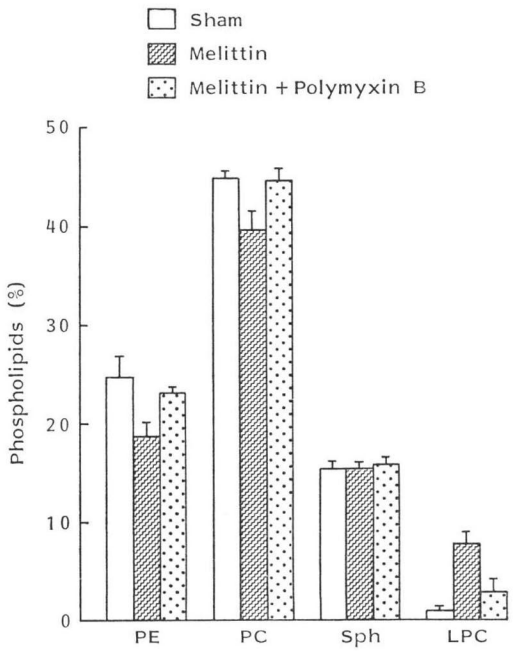
Table 3. Effect of polymyxin B on melittin-induced phospholipid degradation.

Perfusate	Remaining phospholipids after perfusion for 4 hours (μg lipid-phosphorus/g liver)
1) Control	1,327.2±108.7 (100%)
2) Melittin	918.0± 66.5 (69%)
3) Polymyxin B	1,363.6±130.4 (103%)

n=3

Fig. 3. The comparison in phospholipid composition of livers perfused with melittin and polymyxin B with control.

PE, PC, Sph and LPC, respectively stand for phosphatidylethanolamine, phosphatidylcholine, sphingomyelin and lysophosphatidylcholine.



tion in liver slice, the inhibitory effect of polymyxin B was also examined at an organ level by perfusion. Perfusion with Hanks buffer alone did not show any phospholipid degradation. Even perfusion of heat-treated phospholipase A_2 in amounts of 807, 2,727 and 4,035 μg in 50 ml Hanks buffer did not result in phospholipid degradation, although the latter dose should readily hydrolyze 50% of liver phospholipids theoretically *in vitro*. The reason why the exogenous phospholipase A_2 did not work is not clear, but the molecule, MW 29,800, may be too large to pass through the capillary bed. However, by perfusing melittin, the endogenous phospholipids were degradated, and in this system the effect of polymyxin B was examined.

Three 130 g rat livers were perfused separately with 1) Hanks buffer for 30 minutes, 2) Hanks buffer for 5 minutes and then 2 mg melittin in 50 ml Hanks buffer for 20 minutes, and 3) 5 mM polymyxin B solution for 5 minutes and then 2 mg melittin in 5 mM polymyxin B solution in Hanks buffer for 20 minutes. The molar ratio of 2 mg melittin to the endogenous phospholipids in liver slice was about 1: 460, which was smaller than the value, 1: 100, reported by MOLLAY *et al.*[6]. As shown in Table 3, polymyxin B clearly inhibits the melittin-induced phospholipid degradation.

The phospholipid composition of perfused livers were analyzed by thin-layer chromatography. From melittin-treated liver lysophosphatidylcholine was found, but in the control experiment and in the case of melittin plus polymyxin B-treated livers the compound could not be detected (Fig. 3).

Discussion

The tentative hypothesis for pathogenesis of liver cell injury and death induced by hypoxic con-

Fig. 4. The comparison in inhibitory action of antibiotics in term of ID_{50}.

1, Carbenicillin; 2, cephaloridine; 3, chloramphenicol; 4, streptomycin; 5, polymyxin B; 6, peplomycin; 7, tetracaine; 8, EDTA; 9, benzylpenicillin.

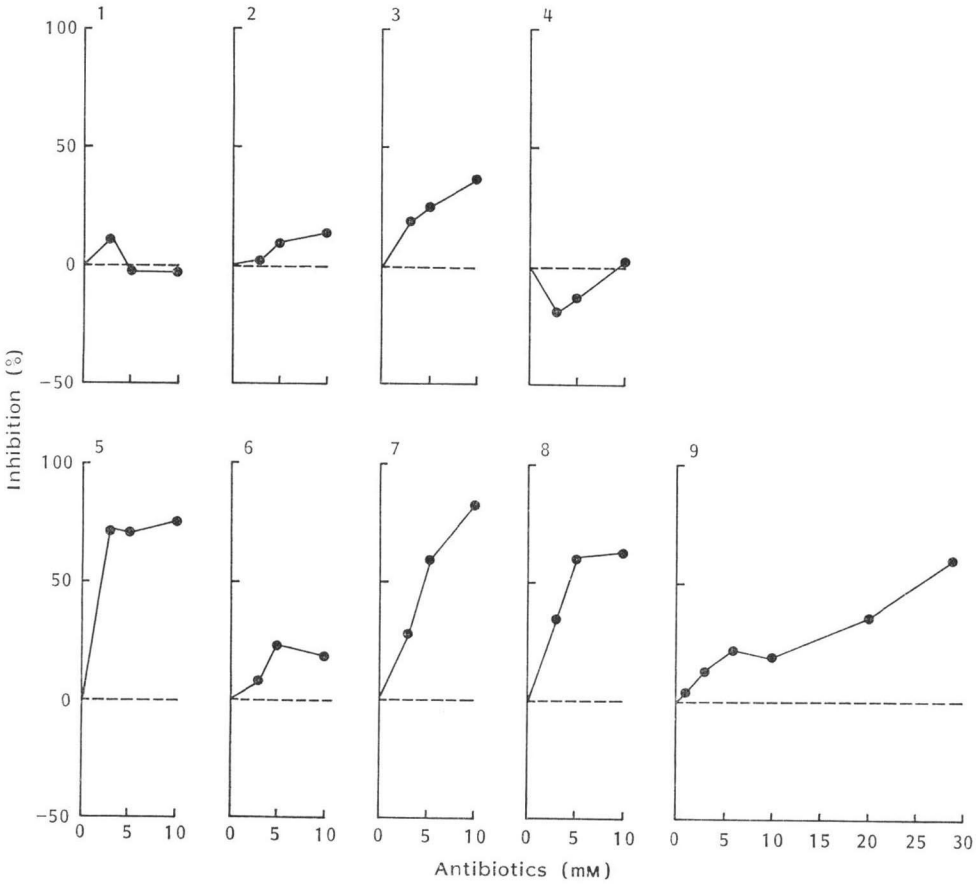


Table 4. Inhibitory effects of some antibiotics on endogenous phospholipid degradation of rat liver and pancreas.

Antibiotics	Liver	Pancreas
Benzylpenicillin	+	+++
Carbenicillin	−	+++
Cephaloridine	−	NT
Tetracycline	NT	+++
Chloramphenicol	+	NT
Streptomycin	−	NT
Polymyxin B	+++	−
Peplomycin	+	NT
EDTA·2Na	++	NT
Tetracaine	+++	+++
Chlorpromazine	NT	+++

NT: Not tested.

ditions reckons with following events: Increase in the cell Ca^{++} content, the activation of endogenous phospholipases, the creation of Ca-channel, the inactivation of mitochondria, the denaturation of structural proteins, and finally cell death[1].

As shown in Fig. 4, some antibiotics tested, particularly polymyxin B, inhibit the endogenous phospholipid degradation of rat liver under hypoxic conditions. The mechanism is certainly not simple, but polymyxin B may perturb the membrane phospholipids as a cationic amphiphilic compound and so disturb the interaction of phospholipases with the biomembrane phospholipids, whereas peplomycin and EDTA inhibit the endogenous phospholipase activity by chelating Ca^{++}, which is essential for phospholipase activity particularly A_2.

Compared to our previous results on pancreas tissue (Table 4), the inhibitory effects of antibiotics on endogenous phospholipase activity were found not to be identical in both tissues, presumably owing to the difference in organ specificity.

The biomedical meaning of antibiotics is originally seen in their actions against microorganisms, but it should be emphasized from the observation mentioned above that some antibiotics prevented the endogenous phospholipid degradation of cells under hypoxic conditions and presumably maintain biological functions of biomembranes of host cells. From the stand point of the latter view, it is very suggestive that dogs, after ligation of their hepatic artery, die of ischemic liver necrosis within 2 or 3 days[12]. However, when they are treated with benzylpenicillin prior to ligation, they survive[13~17].

On the other hand, it also must be noted that aminoglycoside antibiotics inhibit the lysosomal phospholipase A and C in liver[18] and kidney[19], resulting in phospholipid accumulation or aminoglycoside toxicity such as nephrotoxicity. In relation to this, drug-induced lipid storage diseases by, for example, the coronary vasodilator, 4,4′-bis-(diethylaminoethoxy)-α,β-diethyldiphenylethan[20], and amphiphilic cationic drugs[21] are reported and the possible metabolic explanation is the inhibition of phospholipase activity by drugs[21].

Acknowledgments

The authors wish to express their thanks to Dr. I. HONJO in our medical school for his valuable discussion and to Dr. T. OKUMURA in our group for his skillful technical assistance in liver perfusion. This work was supported in part by Grant-in-Aid for Scientific Research from The Ministry of Education, Science and Culture in Japan and also in part by The Naito Foundation Research Grant for 1983.

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