On the Phospholipase Activity in Bovine Seminal Vesicles and Its Possible Role in the Regulation of the Prostaglandin Biosynthesis

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A phospholipid mixture was isolated from bovine seminal vesicle tissue. This phospholipid preparation contained the following prostaglandin precursor fatty acids: 4.7 percent 5,8,11-eicosatrienoic acid, 9.1 percent 5,8,11,14-eicosatetraenoic acid and 0.9 percent 5,8,11,14,17-eicosapentaenoic acid.

With this phospholipid preparation as substrate phospholipase activity could be detected in different fractions of bovine seminal vesicle cells. The highest phospholipase activity was found in the microsomal fraction, less in the mitochondria and essentially no activity in the cytosol.

With a microsome preparation (acetone powder) of bovine seminal vesicles the influence of certain effectors on the phospholipase activity was investigated. Prostaglandins E_2 and $F_{2\alpha}$ (10^{-5} M) showed 19 and 41 percent inhibition, DB-c-AMP (2×10^{-9} M) 54 percent, c-AMP (10^{-9} M) 22 percent, epinephrine (10^{-4} M) 60 percent, testosterone (10^{-5} M) 25 percent inhibition of the phospholipase activity.

Whereas ethanol exhibits also a strong inhibition on the phospholipase activity, it shows a pronounced activation of the prostaglandin synthetase.

The prostaglandins are formed from arachidonic acid (*all-cis*-5,8,11,14-eicosatetraenoic acid), *all-cis*-8,11,14-eicosatrienoic acid and *all-cis*-5,8,11,14,17-eicosapentaenoic acid [1]. These prostaglandin precursors do not occur freely in the tissue but are incorporated in membrane phospholipids [2, 3] and possibly triglycerides [4], from which they must be released by a phospholipase or a triglyceride lipase before being transformed into prostaglandins. It could be possible that the prostaglandin synthesis *in vivo* is controlled by regulating the activity of such a phospholipase or triglyceride lipase.

In this paper experiments are described showing that phospholipase preparations from bovine seminal vesicles are susceptible to the action of certain effectors like DB-c-AMP, c-AMP, norepinephrine, epinephrine, PGE_2 and $PGF_{2\alpha}$ thus indicating a possible role of phospholipases in the regulation of the prostaglandin biosynthesis.

Materials and Methods

All-cis-8,11,14-eicosatrienoic acid methyl ester (NU-Chek-Prep, Elysian, Minnesota, USA), *all-cis*-5,8,11,14-eicosatetraenoic acid (Merck-Schuchardt, München), *all-cis*-5,8,11,14,17-eicosapentaenoic acid methyl ester (Serva, Heidelberg), DB-c-AMP and c-AMP (Boehringer, Mannheim), epinephrine and norepinephrine (Farbwerke Hoechst, Frankfurt), testosterone, tyramine hydrochloride (Merck, Darmstadt). The prostaglandins were a kind gift from Dr. John Pike, The Upjohn Company, Kalamazoo, Michigan, USA.

Gaschromatographic analysis was performed with a Perkin-Elmer B 6/4 gaschromatograph with flame ionization detector and Infotronics CRS-204-integrator.

Phospholipid preparation of BSV: A phospholipid fraction from bovine semical vesicles was prepared according to Rouser *et al.* [5]. Of the total amount of fatty acids present in the phospholipid preparation 4.7 per cent were 8,11,14-eicosatrienoic acid, 9.1 per cent were 5,8,11,14-eicosatetraenoic acid and 0.9 per cent were 5,8,11,14,17-eicosapentaenoic acid as determined by gas chromatography. Qualitative examinations revealed that this phospholipid fraction contained ethanolamine and choline, but no serine. The phospholipid was quantitatively determined by phosphate estimation [6] after degradation of the phospholipid with H_2SO_4/HNO_3.

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Abbreviations: DB-c-AMP, N^6-2′-O-Dibutyryl-c-AMP; PGE, prostaglandin E; BSV, bovine seminal vesicles; GSH, glutathion in the sulfhydryl form; Tris, Tris-hydroxymethylaminomethane.

For the phospholipase incubations the phospholipid was homogenized in a Potter-homogenizer with the appropriate buffer.

Preparation of methyl esters of fatty acids was done according to Metcalfe and Schmitz [7].

Phospholipase preparations

The *acetone powder of BSV* was prepared in the same way as described by Lee and Lands [8] for the preparation of an acetone powder of prostaglandin synthetase.

Isolation of microsomes

The microsomal fraction was prepared according to Yoshimoto *et al.* [9] with the exception that the pellet of microsomes was not washed with Tris-buffer, but suspended in 0.05 M glycine/NaOH-buffer (pH 8.5).

Isolation of mitochondria

The mitochondria were prepared according to common procedures [10]. The crude mitochondria were suspended in 0.25 M sucrose and centrifuged at 24 000 × *g*. The residue was washed and centrifuged as before. This residue (mitochondria plus other large particles) was centrifuged for 120 min at 53 000 × *g* through a gradient of 0.8 – 1.2 M sucrose. The bottom layer (purified mitochondria) was suspended in 0.05 M glycine/NaOH-buffer (pH 8.5).

Cytosol

The supernatant of the centrifugation at 80 000 × *g* for the preparation of microsomes (see above) was used as cytosol-preparation.

Protein determination

Protein was measured by the method of Warburg and Christian [11].

Determination of the phospholipase activity

The phospholipase experiments were carried out as described under the appropriate Tables and Figs (see Results). After incubation the phospholipid was removed from the alkaline solution by extraction with diethyl ether. Then the pH was adjusted to 1 – 2 with 1 N HCl, and the fatty acids were extracted with ether. The ether was evaporated under a stream of nitrogen. In order to remove traces of HCl, the residue was again dissolved in ether and evaporated as before. The fatty acids were kept in a dessiccator *in vacuo* overnight. Then the fatty acids were dissolved in warm ethanol and titrated with 10^{-3} M NaOH in the presence of phenolphtalein (precision ± 2.5%). All values given under Results are corrected for the NaOH-consumption of a blank with ethanol and phenolphtalein. The reproducibility of the values was checked by at least two separate experiments.

Prostaglandin synthetase from BSV was prepared and assayed as described by Yoshimoto *et al.* [9].

Results

In Fig. 1 evidence for phospholipase activity in an acetone powder preparation of BSV is given. This acetone powder was prepared according to the method of Lee and Lands [8] which this authors used as prostaglandin synthetase preparation and which

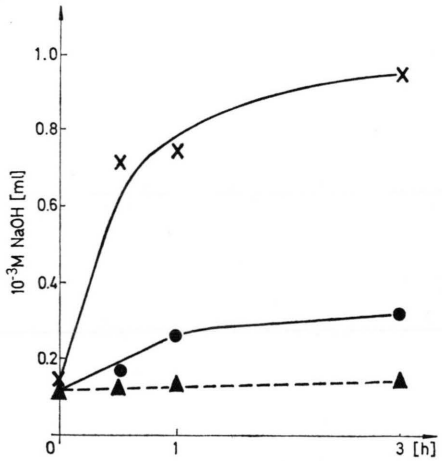

Fig. 1. Time course of the release of fatty acids from BSV-phospholipids by an acetone powder preparation of BSV. Each incubation mixture contained in a total volume of 1.1 ml: 30 mg of BSV acetone powder, 10 μmol of $CaCl_2$, 1 μmol of BSV phospholipid, 110 μmol of glycine-NaOH-buffer (pH 8.5), incubation at 37 °C. (×) + phospholipid, (●) – phospholipid, (▲) – phospholipid, not incubated.

essentially consists of microsomes. As substrate for the experiments of Fig. 1 a phospholipid mixture isolated from BSV was used, but also commercial lecithin is split by the enzyme preparation [12]. From Fig. 1 it can also be seen that the acetone powder still contains endogenous lipids which are split during incubation.

In order to check different compartments of the BSV-cell for phospholipase activity the experiments listed in Table I were carried out. According to Table I the microsomes show the highest phospho-

Table I. Phospholipase activity in different compartments of the bovine seminal vesicle cell. Each incubation mixture contained in a total volume of 1.3 ml: 40 mg of protein, 1 μmol phospholipid, 10 μmol of $CaCl_2$, 150 μmol of glycine-NaOH-buffer (pH 8.5). Incubation at 37 °C for 45 min. From the values the amount of fatty acids released in controls without phospholipid substrates are subtracted.

	Fatti acids released from BSV phospholipids [μmol]
Microsomes	0.50
Mitochondria	0.17
Cytosol	0.06
Acetone powder preparation	0.47

lipase activity, the mitochondria less, and the cytosol contains almost no phospholipase.

It is not surprising that the acetone powder used for the experiments of Fig. 1 exhibits the same phospholipase activity as the microsomes, because this preparation is essentially a microsome preparation (see above).

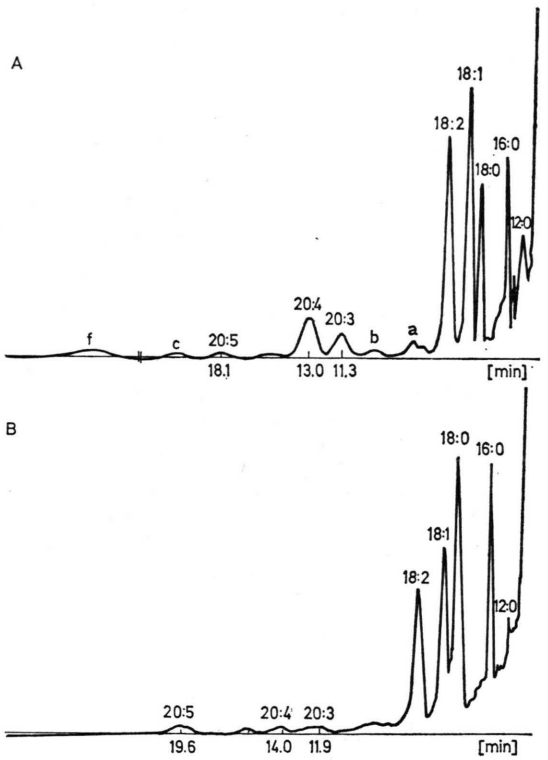

Fig. 2. Gaschromatograms (A) of the total fatty acids of the BSV phospholipids. (B) of the fatty acids released from the BSV phospholipids by the incubation with the acetone powder preparation. Peaks a, b, c and f are unidentified fatty acids.

The fatty acid composition of the BSV-phospholipid mixture used as substrate and the fatty acids released from the BSV phospholipids by the acetone powder preparation are shown in Fig. 2. As expected the action of the phospholipases is nonspecific. The polyunsaturated fatty acids are cleaved, but not preferably.

The fatty acids released in such a phospholipase incubation were incubated with a prostaglandin synthetase preparation from BSV-microsomes according to Yoshimoto *et al.* [9]. Prostaglandins formed from the appropriate precursor acids could be detected.

In these experiments it was also found that ethanol as well as some other solvents have a pronounced enhancing effect on the activity of prostaglandin synthetase. In Table II this effect is demonstrated with arachidonic acid as prostaglandin precursor. Table II shows that also methanol and acetone exhibit this activation but not propanol and butanol.

Table II. Effect of ethanol and some other solvents on the activity of prostaglandin synthetase from BSV. Each reaction mixture contained in a total volume of 1.5 ml: 0.5 mmol of Tris/HCl (pH 8.0), 2.5 μmol of GSH, 0.05 mmol of ascorbic acid, 1.8 μmol of arachidonic acid, 0.646 mg of hemoglobin, 10 mg of microsomal protein from BSV. Incubation 10 min at 30 °C. For experimental details and prostaglandin determination see [9]. The additions of the solvents are in v/v.

	Prostaglandins formed [μg]
Complete	14.4
— arachidonate	0
+ 10% ethanol	28.2
+ 3% methanol	19.7
+ 10% methanol	29.8
+ 20% methanol	20.6
+ 10% methanol added after incubation	14.1
+ 10% acetone	21.5
+ 10% *n*-propanol	0
+ 10% *n*-butanol	0
+ 10^{-3} M NADPH	14.1
+ 10^{-3} M NADH	16.0
+ 1% sodium dodecylsulfate	0
+ 1% Triton X-100	0
+ 1% sodium desoxycholate	0

In order to exclude that this effect is due to a provision of hydrogen from ethanol needed for the prostaglandin formation the experiments with NADH and NADPH were performed. Detergents completely inactivate the prostaglandin synthetase.

In Table III the influence of several substances

Table III. Influence of certain effectors on the phospholipase activity in the acetone powder preparation of BSV. The incubation mixture contained: 110 μmol of glycine-NaOH-buffer (pH 8.5), 10 μmol of $CaCl_2$, 1 μmol of BSV-phospholipid, 30 mg of acetone powder of BSV in a total volume of 1.1 ml. Incubation: 1 h at 37 °C. For further details see Materials and Methods.

	Fatty acids released [μmol]	Inhibition of the phospholipase activity [%]
complete incubation mixture	0.76	
-phospholipid	0.28	
control, not incubated	0.13	(100)
PGE_2 (10^{-5} M)	0.64	19
$PGE_{2\alpha}$ (10^{-5} M)	0.50	41
c-AMP (10^{-9} M)	0.62	22
DB-c-AMP (2×10^{-9} M)	0.42	54
epinephrine (10^{-4} M)	0.38	60
norepinephrine (10^{-4} M)	0.32	69
tyramine (10^{-1} M)	0.72	6
testosterone (10^{-5} M)	0.60	25
ethanol (10% v/v)	0.36	63

on the phospholipase activity in the acetone powder preparation of BSV is shown. All substances tested are more or less inhibitory with the exception of tyramine which does not affect the phospholipase activity even in a very high concentration.

Discussion

It is rather surprising that the regulation of the biosynthesis of such important and pharmacologically very active substances like the prostaglandins is not very much investigated on a molecular basis as far as the release of the precursor acids from the appropriate phospholipid or triglyceride is concerned.

The experiments described in this paper were undertaken in order to get some information on the influence of hormones or pharmacological substances on the phospholipase activity. We used BSV for this research, because this is a tissue readily available and rather active in prostaglandin synthesis.

Experiments on the regulation of the biosynthesis and breakdown of prostaglandins are medically important with regard to the reports that migraine, a severe form of headache may result from a disregulation of prostaglandin production or breakdown [13].

As shown in Table III prostaglandins E_2 and $F_{2\alpha}$ exhibit an inhibitory influence on the phospholipase activity in a concentration of 10^{-5} M. This could reflect a kind of feed-back inhibition, which was also found in other tissues [14, 15]. Also c-AMP and DB-c-AMP are strong inhibitors of the phospholipase action in a concentration of 10^{-9} M. This is in contrast to the situation in the thyroid tissue where c-AMP enhances the production of prostaglandins [4].

Since the experiments of Table III were carried out with an acetone powder preparation the action of the effectors have to be either an effect on the enzyme or on the substrate, *i. e.* on the phospholipid micelles.

We are well aware of the fact that "the fine structure of the lipid-water interface exerts an important influence on the activity of phospholipases" [16]. This fine structure of the lipid-water interface could very well be influenced by high concentrations of such effectors like epinephrine and norepinephrine, which were applied in the concentration of 10^{-4} M. Tyramine even at a concentration of 10^{-1} M has no effect on the phospholipase activity.

Ethanol which has an activating effect on the prostaglandin synthetase (Table II) is rather inhibitory with respect to the phospholipase action (Table III). We assume that also the effect of ethanol is an effect on the micelle structure of the substrate.

As soon as the phospholipases and possibly triglyceride lipases of bovine seminal vesicles can be separated and purified, the regulation of these enzymes can be investigated in more detail. Experiments in order to purify the phospholipases and triglyceride lipases of BSV and also to investigate the regulation of the phospholipase activity using tissue slices and cell culture techniques are presently undertaken.

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