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OCCURRENCE OF SYNERGISM BETWEEN PHOSPHOLIPIDS AND PROSTAGLANDINS IN EXTRACTS OF SOME TISSUES OF COMMERCIAL SQUID

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The phospholipid compositions, prostaglandin-like activities, and ratios of prostaglandins of groups of E, F, and A in prostaglandin extracts of some organs of two species of commercial squid have been investigated.

A series of studies on synergism in the field of prostaglandins (PGs) is known [1-4]. They all describe artificially induced synergism in which concrete compounds have been added to definite PGs and the change in PG-like activity has been determined simultaneously. It was therefore of interest to see how synergism between PGs and phospholipids (PLs) appears in tissues of living organisms.

In an investigation of PG extracts from intact organisms of a number of marine invertebrates we have found a correlation between the degree of PG activity of the extracts and the composition of the coextracted PLs, having revealed those classes of PLs that possess the highest capacity for enhancing this activity [5]. We have also shown that the phenomenon of synergism is characteristic for the PGs and PLs of marine organisms, regardless of their type and habitat [6]. These results confirmed our assumptions of the existence of synergism between PLs and PGs in extracts from an intact organism.

The task of the present work was to show that synergism between the compounds mentioned is also observed in extracts from individual organs and tissues of marine invertebrates. The choice of the object of investigation was not of fundamental importance, and we therefore used the organs and tissues of two species of commercial squid available to us: the skin-free mantle and liver of *Stenoteuthis bartrami*, and also the liver, the skin of the mantle, and the eggs of *Ommatostrephes Sloanei pacificus*.

The results that we obtained showed a relationship between the sets of PLs and the PG-like activities. A particularly sharp and high peak on the kymogram was observed in the biotests of the PGs of an extract of the mantle skin of *O. Sloanei pacificus*. The sets of PGs in this specimen and in the extract of the skin-free mantle of *S. bartrami* were the widest (Table 1).

In the biotest that we used, the PG-like activity of the extracts was due to the presence of PGs of groups E and F. In contrast to *O. Sloanei pacificus*, no PGs of group E were detected in the extracts of *S. bartrami* organs. Consequently, the PG-like activity of these extracts was due to the presence of PGs of group F alone. PGs of group A were detected on the chromatograms of all the samples of both organisms that were investigated, and this was confirmed by the results of alkaline treatment.

The liver and mantle of *S. bartrami* had qualitatively the same PG compositions. The amounts of organic matter and of the PGs of groups E and A in the mantle of this organism were low in comparison with those in the liver (see Table 1); nevertheless, the mantle extract possessed considerable activity, which was shown by a high peak in the kymogram on contraction of the smooth musculature of the rat uterus. In this extract we detected five PGs, and also phosphatidylethanolamine, which exhibits a fairly high degree of synergism [5].

At the same time, the amount of organic matter in the liver of *S. bartrami* was only 1.6 times higher than in the mantle, while the level of PGs of groups E and A was almost 500 times higher. In light of the low activity of the liver extract, this means that the contraction of the uterine musculature is caused by a comparatively small amount of group F PGs. One of

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TABLE 1. Amounts and Compositions of the Phospholipids and Prostaglandins in Extracts of the Organs of *Stenoteuthis bartrami* and *Ommatostrephes Sloanei pacificus*

Object, organ, tissue	Organic matter, % of the weight of the crude tissue	Activity	Presence of PGs			Amount of PGs E+A	Amount of phospholipids, % on the weight of the extract						
			E	F	A		PC	LPC	SM	PE	CAEP	PS	PhG
S. bartrami	0.16	+	–	+	+	0.12	24.50	–	23.44	11.50	7.91	10.36	–
Skin-free mantle	0.26	+	–	+	+	59.50	–	–	8.25	–	–	–	0.08
Liver													
O. Sloanei pacificus	0.56	+	+	+	+	1.23	10.21	–	12.63	4.20	–	–	1.30
Liver	0.18	+	+	+	+	1.90	1.30	29.4	–	18.51	18.11	9.40	–
Mantle skin	0.16	–	–	–	+	13.50	20.20	–	25.10	–	–	–	–
Eggs													

Note: The symbol "–" denotes the absence of the component and of PG-like activity.
Phospholipids: PC) phosphatidylcholine; LPC) lysophatidylcholine; SM) sphingomyelin; PE) phosphatidylethanolamine; CAEP) ceramide aminoethylphosphonate: PS) phosphatidylserine; PhG) phosphatidylglycerol.

the two PLs detected in the liver extract, phosphatidylglycerol, exhibits the highest degree of synergism [5]. It is possible that the PG F present in very small amounts in the *S. bartrami* liver was also activated by PhG.

Work with natural mixtures of bioactive compounds is not so simple and unambiguous as in the case of artificial sets of PGs and PLs. This is probably connected with the fact that model systems are simpler and do not take into account many factors acting in the tissues of living organisms and permitting a multiplicity of routes to the solution of one and the same question.

EXPERIMENTAL

The animals were trapped in the Komandorskie islands in the summer-autumn period.

Freshly excised organs of the squids were fixed with acidified ethanol. Extracts of the PGs in mixtures with accompanying compounds were obtained by a method described previously [7]. The organic matter in them was determined by the method of [8]. The quantitative estimation of the PGs was made by UV spectroscopy [9]. PG-like activity was determined by biotests on the smooth musculature of the rat uterus [10]. To separate and identify the PGs of groups E, F, and A we used TLC on Silufol UV-254 (Czechoslovakia) in the following systems: benzene–dioxane–acetic acid (20:20:0.5) and benzene–ethyl acetate–formic acid (75:25:1) [11, 12].

As comparison specimens we used PGA_2 (Serva) and the well-studied PGs of the soft coral *Plexaura homomalla*. To reveal the spots, the plates were treated with a 3% solution of copper acetate in 15% aqueous phosphoric acid, followed by heating at 100°C for 10 min [13]. In addition to TLC, for identifying the PGs we used UV spectrometry with alkaline treatment of the extracts [9].

The PLs were separated by two-dimensional micro-TLC in the following systems: chloroform–methanol–28% ammonia (65:25:5) (first direction) and chloroform–acetone–methanol–acetic acid–water (30:40:10:10:5) (second direction) [14], with revelation by the molybdate reagent [15]. Choline-containing compounds were detected with the Dragendorff reagent, and compounds having a free amino group with a solution of ninhydrin in acetone.

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