ACTA CHEMICA SCANDINAVICA 17 (1963) S 282 – S 287

Isolation of Prostaglandin E_1 from Calf Thymus

Prostaglandins and Related Factors 20

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Lipid soluble, acidic material with smooth muscle stimulating activity has been extracted from calf thymus and separated by different chromatographic techniques. Prostaglandin E_1 was identified as the major biologically active component. The concentration of this factor, determined by isotope experiments was found to be 0.8 μg per g tissue.

The presence of a vasodepressor and smooth muscle stimulating activity in seminal plasma and in sheep vesicular gland was first reported by Goldblatt and von Euler[1,2]. The active principle was called prostaglandin by Euler, who demonstrated that it was due to a lipid soluble factor that Theorell with his electrophoresis apparatus showed to be acidic[3–6].

Two crystalline compounds, prostaglandin E_1 and $F_{1\alpha}$*, were later isolated from sheep vesicular gland by Bergström and Sjövall[7]. The structures of these compounds have been reported[8] (Fig. 1). Later, two new biologically active compounds, prostaglandin E_2 and E_3, were isolated from the same source[9]. The structure of prostaglandin E_2 has also been elucidated (Fig. 1). Recently, a biologically active reduction product of prostaglandin E_2, called prostaglandin $F_{2\alpha}$, was isolated in small amounts from lungs of sheep and pig[10] (Fig. 1). Furthermore, the same compound was later identified in extracts of sheep iris[11]. The present report is concerned with isolation of smooth-muscle stimulating lipid soluble material from calf thymus, which has led to the identification of prostaglandin E_1.

EXPERIMENTAL

Extraction. Calf thymus glands (10 kg) were collected immediately after slaughter and kept at −20°C until being processed. The glands were minced in a semi frozen condition and stirred

* Nomenclature: The systematic nomenclature is based on the trivial name prostanoic acid for the parent C_{20} acid numbered as shown in Fig. 1. Prostaglandin E_1 (earlier PGE): 11α, 15-dihydroxy-9-keto-prost-13-enoic acid. Prostaglandin E_2: 11α, 15-dihydroxy-9-keto-prosta-5, 13-dienoic acid. Prostaglandin $F_{1\alpha}$ (earlier PGF_1 of PGF_{1-1}): 9α, 11α, 15-trihydroxy-prost-13-enoic acid. Prostaglandin $F_{2\alpha}$ (earlier PGF_{1-2}): 9α, 11α, 15-trihydroxy-prosta-5, 13-dienoic acid.

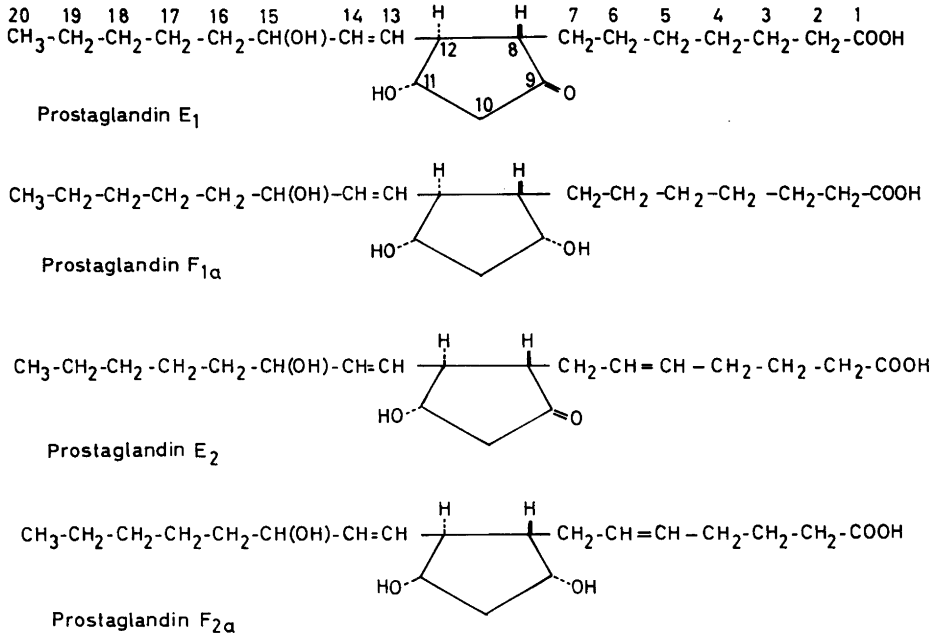

Fig. 1.

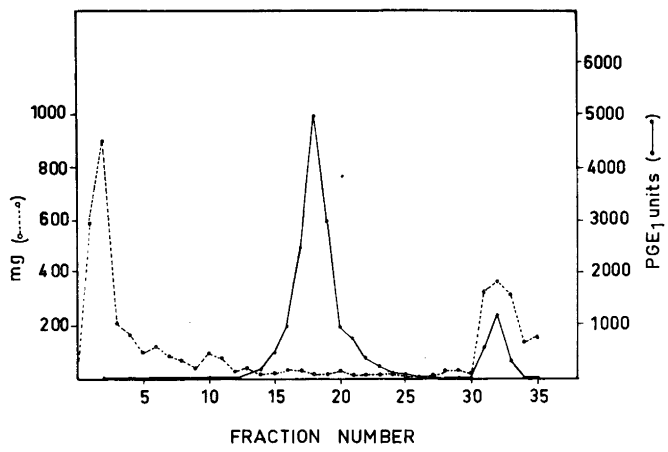

Fig. 2. Silicic acid chromatography of acidic lipids from calf thymus. Column: 550 g silicic acid. Fractions: 1600 ml. 1–10 Ethylacetate-hexane (30 : 70). 11–20 Ethylacetate-hexane (70 : 30). 21–30 Ethylacetate. 31–35 Methanol.

mechanically with 40 l of 96 % ethanol for 4 h. The suspension was filtered and the filtrate and washings concentrated by evaporation *in vacuo*. The resulting aqueous solution was acidified to pH 3 and extracted with ethylacetate. The combined ethyl acetate extracts were washed with a phosphate buffer pH 8 followed by acidification of the buffer to pH 3 and extraction with ethylacetate. The residue obtained after evaporation of the ethylacetate solution was subjected to a three stage distribution between equal volumes of petroleum ether and ethanol-water (2 : 1). The combined aqueous phases were evaporated to dryness.

The biological activities of the extracts were determined on duodenal strips of rabbits against a standard of prostaglandin E_1. (One unit of prostaglandin E_1 is equal to the effect of 1 μg of crystalline prostaglandin E_1).

Silicic acid chromatography. Material present in the aqueous ethanol extract (11 g) was separated on a column of 550 g silicic acid (Mallinckrodt, activated at 120°C (Fig. 2) as described previously[12].

Reversed phase partition chromatography. The residue (231 mg) obtained by evaporation of fractions 14–23 of the chromatography shown in Fig. 2 was separated by reversed phase partition chromatography (Fig. 3) on 45 g of hydrophobic SuperCel using the solvent system described earlier[9].

Thin layer chromatography (TLC). The biologically active material, purified by partition chromatography, was subjected to thin layer chromatography both on an analytical and preparative scale according to the procedure described by Green and Samuelsson[13].

RESULTS

Material obtained from calf thymus by ethanol extraction was shown in preliminary experiments to possess smooth-muscle stimulating activity. The crude extract from 10 kg of glands was subjected to fractionation (see experimental) into acidic and neutral lipids. By following the biological activity in all steps it was demonstrated that the smooth-muscle stimulating material was almost exclusively confined to the acidic fraction. This material could be further purified by distribution between petroleum ether and aqueous ethanol, which resulted in

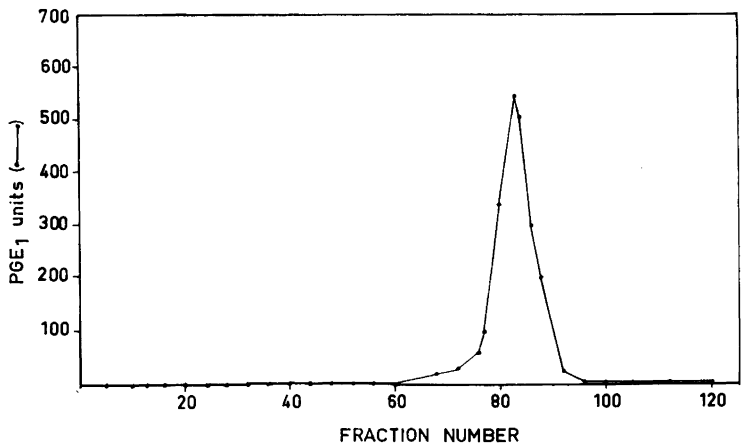

Fig. 3. Reversed phase partition chromatography of material eluted in fractions 14–23 of the chromatography shown in Fig. 2. Column: 45 g Hydrophobic Hyflo SuperCel. Fractions: 17 ml. Stationary phase: 40 ml isooctanol-chloroform (1 : 1). Moving phase: 47.5 % aqueous methanol.

removal of the bulk of relatively non-polar lipids whereas the biologically active material was completely recovered in the aqueous phase.

Material purified in this way was separated by silicic acid chromatography. The elution sequence applied has been worked out earlier for isolation of prostaglandins and offers the possibility of separating prostaglandins of the PGE series from those of the PGF series[12].

Smooth-muscle stimulating material was eluted with ethylacetate-hexane (70 : 30) and with ethylacetate in the same manner as would be expected for prostaglandins of the PGE type on this size of column. No PGF compounds were detected. Elution of the column with methanol gave a second peak with smooth-muscle stimulating material of more polar nature. The latter material has not been further characterized.

Reversed phase partition chromatography of fractions 14–23 from the silicic acid chromatography yielded one peak (Fig. 3) with a retention volume characteristic of prostaglandin E_1. This chromatogram also shows the absence of any of the other known prostaglandins, which would separate from PGE_1 under the conditions employed.

Thin layer chromatography of material present in 1300–1550 ml effluent of the chromatography in Fig. 3 showed one spot with the same R_F-value as PGE_1 and at least two spots with higher R_F-values. Purification by preparative TLC of part of the material from the reversed phase partition chromatography gave a semicrystalline substance, which was pure and identical with prostaglandin E_1 as judged by thin layer chromatography both of the methyl ester and the free acid. Determination of the biological activity showed within the errors of the method the same activity on a weight basis for the isolated compound and for crystalline prostaglandin E_1.

Compounds of the PGE series give rise to an UV absorption with λ_{max} at 278 mμ on treatment with 0.5 N sodium hydroxide at room temperature. This chromophore is due to a dienone formed by dehydration and isomerization of the double bond produced. Treatment of 40 μg of the isolated material with 0.5 N sodium hydroxide solution gave the same absorbancy at 278 mμ as 40 μg prostaglandin E_1 treated under identical conditions.

Further proof of the identity of the isolated compound with prostaglandin E_1 was obtained by mass spectrographic analyses of the methyl ester. This proved practically identical with that of the methyl ester of prostaglandin E_1.

A more accurate estimation of the amounts of prostaglandins in tissue extracts of this type has been greatly hampered by the sensitivity of the compounds and by the rather involved processes, which are necessary for their isolation.

However, by the availability of tritium labelled prostaglandin E_1[14], it was possible to follow the losses occurring during the isolation by isotope dilution experiments. In another experiment, carried out essentially as described above, tritium labelled prostaglandin E_1 (80 μg; 50×10^6 cpm/mg) was added at the beginning of the ethanol extraction. Determination of the total radioactivity coinciding with the peak of biological activity in the reversed phase partition chromatography showed a yield of approximately 42 per cent at this stage of

the purification. The total amount of prostaglandin E_1, based on determination of the biological activity of this material and corrected for the losses during isolation, was found to be 0.8 μg per g tissue (wet weight). Essentially the same figure was obtained when this procedure was repeated on material further purified by thin layer chromatography.

DISCUSSION

The present work demonstrated unequivocally the presence of prostaglandin E_1 in thymus. It is also evident that prostaglandin E_1 is the major prostaglandin present in the material examined, since the other known derivatives would have been detected by the methods applied. The amount of prostaglandin E_1 present in thymus was determined by adding tracer amounts of tritium labelled prostaglandin E_1 at the beginning of the isolation and determining the biological activity and radioactivity of isolated material. This method gave a concentration of 0.8 μg per g tissue (wet weight).

The isolation of prostaglandin E_1 from calf thymus affords a third example of the occurrence of prostaglandins in organs other than those primarily concerned with the genital functions. Recently, prostaglandin $F_{2\alpha}$, a reduction product of prostaglandin E_2 (Fig. 1), was isolated from lungs of sheep and pig[10] and later from sheep iris[11]. The concentration of prostaglandin in sheep lung, determined with the same technique as in these experiments, was found to be about 0.5 μg per g tissue[11]. Judging from the activity in crude fractions similar amounts are present in both male and female lungs of sheep, cattle and horse. As a comparison it might be mentioned that the concentration in human seminal plasma is considerably higher, *i. e.* the sum of the various prostaglandins present exceeded 50 μg per ml[12].

It must also be noticed that two of the species mentioned above in which prostaglandins have been found in lungs and thymus do not appear to have prostaglandins in their sperm or accessory genital glands.

Even if the prostaglandins might have an important function in the genital sphere of humans and sheep these facts indicate that prostaglandins might have a more general physiological function.

This contention is further supported by the very high biological activity shown by the different prostaglandins. Thus, the threshold dose of prostaglandin E_1 on rabbit duodenum is 0.003–0.01 μg per ml[15] and an infusion of 0.2–0.7 μg/kg/min of prostaglandin E_1 in humans causes a fall in blood pressure[16] and an increased heart rate and it can counteract in equimolar amounts the action of catecholamines on both blood pressure and release of free fatty acids[17].

Further work is needed to clarify the physiological role of this new group of hormonally active compounds that seems to be widely distributed in animal tissues.

This work is part of investigations supported by *Statens Medicinska Forskningsråd* and *Stiftelsen Therese och Johan Anderssons Minne.*

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Received April 9, 1963.