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THE IDENTIFICATION OF PROSTAGLANDINS IN HUMAN UMBILICAL CORD

BY

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The smooth muscle stimulating action of extracts of human umbilical cord vessels has been recently reported from this laboratory (Karim 1966a, b). On the basis of these previous investigations it was suggested that the smooth muscle contracting activity of the cord extract was due to an unsaturated hydroxy acid. Further work, however, has revealed the presence of several such factors in this tissue. This report describes the identification of prostaglandins E_1 E_2 $F_{1\alpha}$ and $F_{2\alpha}$ in extracts of umbilical blood vessels. (see Bergström & Samuelsson (1965) for the chemistry of these substances). A preliminary account of this finding was reported to the British Pharmacological Society Meeting in July 1966.

METHODS

Umbilical arteries and veins isolated from approximately 80 fresh cords were split along their longitudinal axis and washed with distilled water to remove the blood. The blood vessels (1,000 g), after drying between filter paper were homogenized and extracted with 4 ml. of absolute alcohol/g tissue. The mixture after standing for 24 hr at 4° C was filtered. The filtrate was evaporated to 100 ml. at 40° C under reduced pressure. The prostaglandins were extracted by a slightly modified method of Samuelsson's (1963). The solution (pH 6.5–7) was shaken with an equal volume of diethyl ether. The ether phase contained less than 1% of the total biological activity and was discarded. The aqueous phase was acidified to pH 3 with N hydrochloric acid and extracted three times with equal volumes of diethyl ether. The ether phase contained 95% of the biological activity, it was evaporated to dryness and the residue was partitioned between equal volumes of petroleum ether (b.p. 40° C–60° C) and 66% ethanol. The aqueous-ethanol phase after evaporating to one-fifth of the volume was extracted three times with equal volumes of diethyl ether at pH 3.

Silicic acid chromatography

The silicic acid (Mallinckrodt) was activated at 110° C for 1 hr before use. Column (20 cm long, 1.25 cm internal diameter) was packed by making a slurry of 10 g activated silicic acid with ethyl acetate: benzene (30:70). The residue of the evaporated ether extract was fractionated on this column with the elution sequence shown in Fig. 1.

Thin layer chromatography

This was performed as described by Green & Samuelsson (1964). The glass plates (0.4 cm.×20×20 cm) were coated by spreading a mixture of 30 g silica gel G and 60 ml. distilled water using the Stahl applicator (0.25 mm thickness). In some experiments silver nitrate (1 g/30 g adsorbent) was dissolved in the water before addition to the silica gel. The plates were activated for 30 min at 110° C before use. The material to be analysed was applied as spots or as bands. The active materials on the plate after development were either visualized by spraying with 10% phosphomolybdic acid in ethanol followed by heating at 110° C for 15 min or for preparative thin layer chromatography the zones at 1 cm intervals the starting line to the solvent front, except at the zones

corresponding to the prostaglandin spots on the marker plates, were scraped off and extracted as described by Green & Samuelsson (1964). The following solvent systems described by these authors were used:

A I. Benzene—Dioxan—Acetic acid. 20:20:1.

AII. Ethyl acetate—Acetic acid—Methanol—2,2,4-trimethylpentane—Water. 110:30:35:10:100.

MII. Ethyl acetate—Methanol—Water. 8:2:5.

Solvent systems AII and MII were equilibrated for 2 hr and the upper phase used. Methyl esters of prostaglandins were prepared by treatment of free acids with diazomethane.

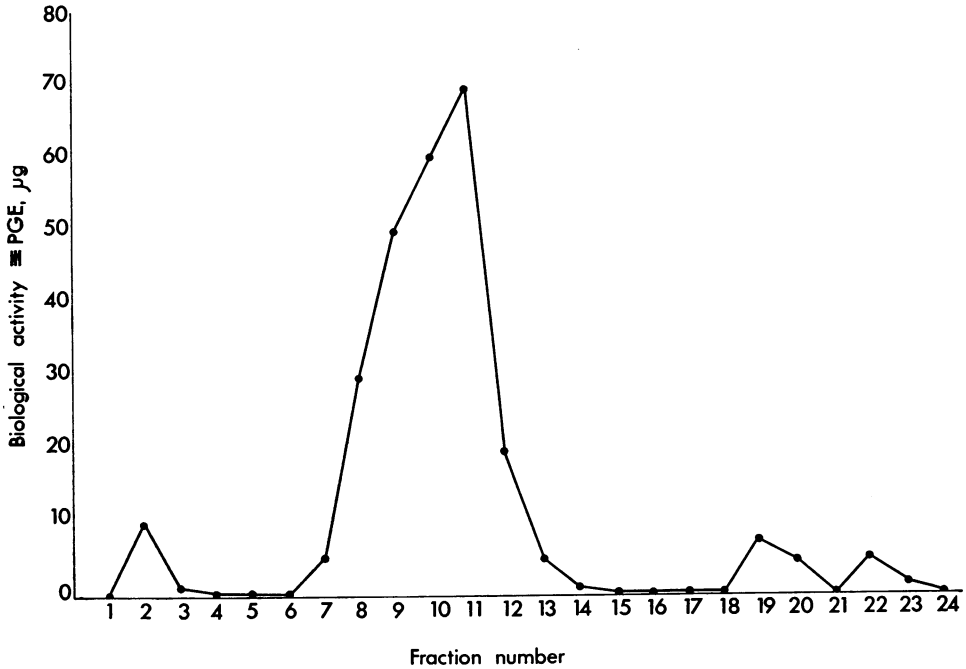

Fig. 1. Separation of ether purified extract of human umbilical cord vessels by silicic acid chromatography. Column 10 G ; Fractions 100 ml. Ethyl acetate—benzene was used as the eluent in the following ratios: Fractions 1–4, 30:70 ; Fractions 5–15, 60:40 ; Fractions 17–21, 80:20 ; Fractions 22–25, methanol. Biological activity was determined on the isolated guinea-pig proximal colon preparation against a pure sample of PGE_1.

Extracts of placental vessels. The large arteries and veins on the surface of the placenta were separated from the rest of the organ and extracted in the same way as the cord vessels.

Extracts of placental tissue. The chorionic membrane and the amnion were separated from a fresh placenta. The large blood vessels on the surface of the foetal side of the placenta were removed and discarded. Two or three cotyledons were separated from the rest of the placenta and extracted in the same way as the cord vessels.

Foetal and maternal blood

Pooled samples of maternal and foetal plasma (100 ml. each) were separately extracted by the method described by Samuelsson (1963) for the extraction of prostaglandins from seminal plasma.

Isolated smooth muscle preparations

Guinea-pig proximal colon was set up in a 5 ml. organ bath of Tyrode solution at 32° C gassed with oxygen. The isotonic contractions were recorded with a frontal writing lever on a smoked drum. A dose cycle of 10 min was used with 2½ min contact time.

Isolated umbilical artery preparation

Approximately 4–5 cm of umbilical artery isolated from fresh cord was split open along its longitudinal axis and suspended in Krebs solution at 37° C in a 2 ml. bath gassed with oxygen: carbon dioxide (95:5). The isometric contractions were recorded with a tensile isometric transducer and an ink-writing pen recorder. The dose cycle of 15–20 min with 5 min contact time was used.

With all the following preparations longitudinal contractions were recorded isotonically with a frontal writing lever on a smoked drum; the bath volume was 5 ml.

Rabbit jejunum. Proximal jejunum, from rabbits weighing 1.5–2.0 kg was suspended in Tyrode solution at 30° C gassed with air.

Hamster colon. The ascending colon, from hamsters weighing 100–150 g was suspended in de Jalon solution at 36° C gassed with air.

RESULTS

Because of its high sensitivity guinea-pig proximal colon has been used for the routine bioassay of prostaglandins extracted from biological tissues and fluids. The responses of this preparation to the four prostaglandins isolated from the umbilical blood vessels are shown in Fig. 2.

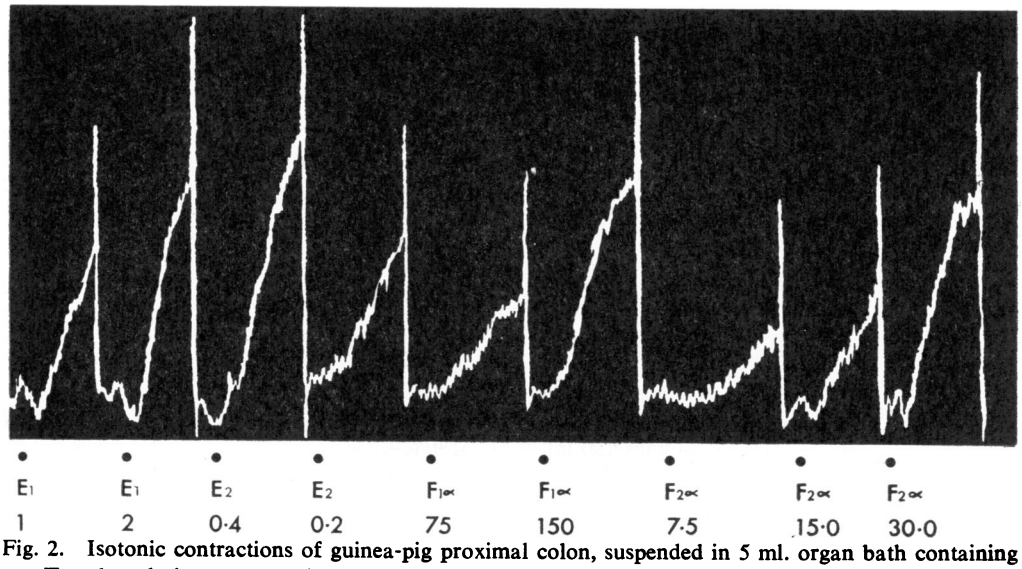

Fig. 2. Isotonic contractions of guinea-pig proximal colon, suspended in 5 ml. organ bath containing Tyrode solution gassed with oxygen. E_1 = prostaglandin E_1, E_2 = prostaglandin E_2, $F_{1\alpha}$ = prostaglandin $F_{1\alpha}$ and F_{α} = prostaglandin $F_{2\alpha}$. Figures indicate concentrations of different prostaglandins in ng/ml. Dose cycle 10 min, contact time 2½ min.

Silicic acid chromatography

Fractionation of the final ether extract of umbilical cord vessels on silicic acid column resulted in four peaks of biological activity (Fig. 1). The main part of the biological activity was present in peaks 2 and 3 eluted with ethyl acetate: benzene (60:40 and 80:20 respectively). With the aid of tritium labelled materials, Samuelsson (1963) has shown that prostaglandins of E group can be eluted from silicic acid column with ethyl acetate: benzene 60:40 and those of the F series with ethyl acetate: benzene 80:20. Materials from peaks 2 and 3 were therefore further examined for the presence of prostaglandins Es and Fs respectively.

Identification of E prostaglandins

Fractions 6–14 were pooled and chromatographed on glass plates coated with silica gel G in solvent system AI which separates prostaglandins E from prostaglandins F. All the biological activity was recovered from the zone corresponding to E prostaglandins (R_f 0.60–0.64). The material recovered from this zone was further chromatographed on thin layer plates of silica gel containing silver nitrate using solvent system AII. By this method the biological activity was recovered from the zones corresponding to PGE_1 (R_f 0.78–0.82) and PGE_2 (R_f 0.70–0.74). Part of the material was converted into methyl esters and chromatographed on silica gel plate containing silver nitrate in solvent system MII. When the plates were sprayed with phosphomolybdic acid two blue spots appeared with R_f values identical with those of the methyl esters of PGE_1 (R_f 0.65–0.68) and PGE_2 (R_f 0.56–0.59) respectively.

Indentification of F prostaglandins

When the material from peak 3 (fractions 18–21) of silicic acid column was chromatographed on plates coated with silica gel in solvent system AI all the biological activity was recovered from the zone corresponding to F prostaglandins (R_f 0.44–0.48). With further thin layer chromatography of this material on silica gel plates containing silver nitrate in solvent system AII the biological activity was found in the same region as pure prostaglandins ($F_{1\alpha}$ (R_f 0.62–0.66) and $F_{2\alpha}$ (R_f 0.44–0.48) respectively. Methyl esters of this material run in solvent system MII had identical R_f values to those of the corresponding methyl esters of $PGF_{1\alpha}$ (R_f 0.47–0.50) and $PGF_{2\alpha}$ (R_f 0.36–0.39). In all these experiments a marker plate with pure prostaglandins was run simultaneously and the guinea-pig proximal colon used for the biological activity.

Further proof regarding the identity of the four substances isolated from umbilical cord extracts was obtained by parallel biological assays of the separated materials on three different preparations against prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$. Within the limits of error of biological assays the activity of the four prostaglandins from umbilical cord vessels were the same on all the three preparations (Table 1). Distribution of the four prostaglandins is shown in Table 2.

TABLE 1

ASSAY OF FOUR PROSTAGLANDINS ISOLATED FROM UMBILICAL BLOOD VESSELS ON THREE DIFFERENT BIOLOGICAL PREPARATIONS

Pure prostaglandins PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$ respectively were used as standards.

Mean ± standard error.

Material separated by thin layer chromatography on plates containing 5% $AgNO_3$ in solvent system AII	Bioassay preparation		
	Guinea-pig proximal colon	Rabbit jejunum	Hamster colon
1 Fraction with R_f value identical with PGE_1	40 μg PGE_1 ± 2·2	35 μg PGE_1 ± 3·1	36 μg PGE_1 ± 4·1
2 Fraction with R_f value identical with PGE_2	30 μg PGE_2 ± 4	31 μg PGE_2 ± 2·9	40 μg PGE_2 ± 4·3
3 Fraction with R_f value identical with $PGF_{1\alpha}$	100 μg $PGF_{1\alpha}$ ± 5·6	90 μg $PGF_{1\alpha}$ ± 8·4	94 μg $PGF_{1\alpha}$ ± 6·2
4 Fraction with R_f value identical with $PGF_{2\alpha}$	80 μg $PGF_{2\alpha}$ ± 3·4	100 μg $PGF_{2\alpha}$ ± 6·2	94 μg $PGF_{2\alpha}$ ± 7·4

TABLE 2

DISTRIBUTION OF SMOOTH MUSCLE STIMULATING ACTIVITY DURING FRACTIONATION OF THE EXTRACTS OF HUMAN UMBILICAL CORD AND PLACENTAL VESSELS

The biological activity was determined on the isolated guinea-pig proximal colon preparation. The amounts refer to the total prostaglandin recovered at each stage of the extraction procedure

Source	Biological activity ≡ PGE_1 μg: 1,000 g cord vessels (μg)	Biological activity ≡ PGE_1 μg: 200 g placental vessels (μg)
Ethanol extract	250	40
Partition with ether:		
(a) Neutral pH		
(i) Ether phase	1	0·5
(ii) Aqueous phase	240	45
(b) Acid pH (pH 3)		
(i) Ether phase	250	·42
(ii) Aqueous phase	5	1
Partition with petroleum ether:		
Petroleum ether	No activity	No activity
Aqueous ethanol	250	40
Silicic acid chromatography:		
Ethyl acetate–benzene 30 : 70	10	1
Ethyl acetate–benzene 60 : 40	210	35
Ethyl acetate–benzene 80 : 20	8	2·5
Methanol	6	2·0
Thin layer chromatography (Solvent system AI followed by AII)		
PGE_1	40	8
PGE_2	150 (≡30 μg PGE_2)	24 (≡6 μg PGE_2)
$PGF_{1\alpha}$	1 (≡100 μg $PGF_{1\alpha}$)	0·3 (≡30 μg $PGF_{1\alpha}$)
$PGF_{2\alpha}$	6 (≡80 μg $PGF_{2\alpha}$)	1·5 (≡20 μg $PGF_{2\alpha}$)

Extracts of placental vessels

Extracts of placental vessels fractionated on silicic acid column followed by thin layer chromatography were found to contain the four prostaglandins identified in the extracts of cord vessels (Table 2). Placental tissue (excluding the large surface vessels) contained no detectable activity (less than the equivalent of 0.25 ng PGE_1/g). Maternal plasma (100 ml.) and foetal plasma (100 ml.) extracts contained less than the equivalent of 0.05 ng PGE_1/ml.

Effect of prostaglandins on the isolated umbilical artery preparation

The typical effect of four prostaglandins on the isolated umbilical artery preparation are shown in Fig. 3. Prostaglandins E_2, $F_{1\alpha}$ and $F_{2\alpha}$, were qualitatively similar; E_2 and $F_{2\alpha}$ were found to be about equiactive, but $F_{1\alpha}$ was less active in producing the contraction of the preparation. Prostaglandin E_1 caused the umbilical artery to relax. A mixture of the four prostaglandins in the proportion in which they are present in the cord vessels always produced contraction of the isolated umbilical artery preparation.

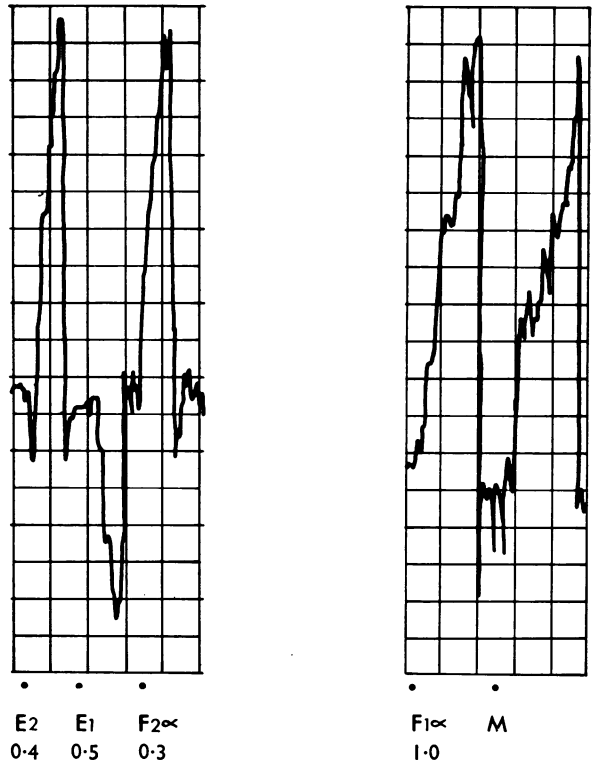

Fig. 3. Isometric contractions of the isolated umbilical artery preparation suspended in a 2 ml. organ bath of Krebs Ringer solution gassed with oxygen: CO_2 (95:5). E_1 = prostaglandin E_1; E_2 = prostaglandin E_2; $F_{1\alpha}$ = prostaglandin $F_{1\alpha}$; $F_{2\alpha}$ = prostaglandin $F_{2\alpha}$. At M a mixture of the four prostaglandins in proportion found in the umbilical cord vessels was injected (E_1 = 0.1 μg/ml.; E_2 = 0.075 μg/ml.; $F_{1\alpha}$ = 0.25 μg/ml.; and $F_{2\alpha}$ = 0.2 μg/ml.). Figures indicate concentrations of different prostaglandins in μg/ml. Dose cycle 20 min, contact time 5 min.

DISCUSSION

Extracts of human umbilical vessels contract the guinea-pig isolated proximal colon (Karim, 1966a, b). This preparation, described by Botting (1965) for the assay of vasopressin, also contracts in the presence of acetylcholine (100 ng/ml.), histamine (100 ng/ml.), 5-hydroxytryptamine (500 ng/ml.), angiotensin (10 ng/ml.) and bradykinin (5 ng/ml.), but none of these substances is responsible for the activity found in umbilical cord extracts. Most of the activity has been shown to be due to four prostaglandins, identified as prostaglandins E_1, E_2, $F_{1\alpha}$ and $F_{2\alpha}$ by their chromatographic behaviour and biological activity.

The identification of prostaglandins in the umbilical cord vessels raises the question of their origin. Recent observations indicate that the placenta is less of a barrier to the passage of drugs than is generally thought (Baker, 1960). Most of the substances in the maternal blood could thus have an access to the umbilical circulation (Moya & Thorn-

dyke, 1962). However, the absence of prostaglandins in the maternal and foetal blood at term would tend to suggest that these substances are not derived from the maternal circulation but are formed within the uterine cavity.

It is now well established that the cells of the placenta can synthesize biologically active substances such as progesterone and oestrogens (Stewart, 1951; Zander, 1959). The absence of prostaglandins from the placental tissue (except the large surface vessels) excludes the possibility that these substances are formed in the placenta.

The presence of prostaglandins in the human amniotic fluid obtained during labour has been recently reported (Karim, 1966c). It is conceivable that the prostaglandins in the umbilical cord and placental vessels originate from the amniotic fluid. This could happen in one of two ways: first, that the foetus at term has been calculated to swallow 450 ml. of amniotic fluid/24 hr (Pritchard, 1965). However, the absence of prostaglandins in cord blood at term tends to suggest that the prostaglandins in ingested amniotic fluid are either destroyed or metabolized before they reach the cord. The second possibility is that the prostaglandins from the amniotic fluid get across the umbilical cord and into the umbilical blood vessels. Plentl (1961) in perfusion experiments has shown that a lively exchange of water and metabolites takes place across the walls of the cord.

The identification of prostaglandins in the umbilical cord vessels indicates further the distribution of these substances outside the genital sphere. The discovery of these substances in the umbilical and placental vessels also raises the question of their possible physiological role. Because of the action of prostaglandins E_2, $F_{1\alpha}$ and $F_{2\alpha}$ in causing the isolated umbilical artery preparation to contract, it is conceivable that they play a role in the closing of the cord vessels after the foetus is expelled. Further work that is now in progress to investigate the distribution of prostaglandins in umbilical cord vessels and amniotic fluid at different stages of pregnancy may help to answer this question.

SUMMARY

1. The smooth muscle contracting activity of the extract of human umbilical blood vessels has been shown to be due to several closely related factors.

2. Four of these fractions have been identified as prostaglandins E_1, E_2, $F_{1\alpha}$, and $F_{2\alpha}$.

3. The possible origin of prostaglandins and their physiological function in closure of the umbilical blood vessels is discussed.

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