Preliminary Communications

Isolation of Prostaglandin E₁ from Human Seminal Plasma

PROSTAGLANDINS AND RELATED FACTORS 11

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(Received for publication, June 21, 1962)

The pharmacodynamic effects of lipid-soluble material in human seminal plasma and male accessory glands were discovered by Goldblatt (1) and von Euler (2), who independently found that this material stimulates different smooth muscle organs and that it contains a blood-pressure-lowering principle. Further studies have been pursued by Eliasson (3). Two active compounds (prostaglandins E₁ and F₁) were isolated in crystalline form from the vesicular glands of sheep by Bergström and Sjövall (4). These factors both have smooth muscle-stimulating actions, whereas only prostaglandin E₁ has a strong blood pressure-reducing activity (5, 6). Their chemical structures (Fig. 1) have recently been elucidated (7). Further work has shown the presence in sheep prostate glands of two related compounds, prostaglandin E₂ and E₃ that contain, respectively, one and two additional double bonds (8). A factor with a similar chemical structure (Fig. 1) has recently been isolated from normal sheep and swine lung (9). This report is concerned with the separation of smooth muscle-stimulating factors in human seminal plasma, one of which has been found to be identical with prostaglandin E₁.

Samples of human seminal plasma were obtained from the Clinics of Obstetrics and Gynecology at Karolinska Sjukhuset and Sabbatsbergs Sjukhus, Stockholm, through the courtesy of Drs. E. Nordlander and L. Lagergren. The samples were added to ethanol and stored in a deepfreeze at -20°. A volume of 200 ml of seminal fluid in 600 ml of ethanol was used in the experiments described here.

After the precipitated material had been removed by filtration and washed with ethanol, the filtrate and the washings were evaporated under reduced pressure to a volume of 10 ml. This solution was diluted with 20 ml of water, acidified with N hydrochloric acid to pH 3, and extracted three times with equal volumes of ether. The combined ether extracts were washed with water until there was a neutral reaction, dried over sodium sulfate, and evaporated to dryness under reduced pressure. The residue (182 mg) was partitioned three times between equal volumes of 60% (by volume) aqueous ethanol and light petroleum. The two phases were evaporated to dryness and stored in a deepfreeze at -20°. A volume of 200 ml of seminal fluid in 600 ml of ethanol was used in the experiments described here.

The physiologically active material was separated into two peaks (Fig. 2). The material present in 530 to 750 ml (Fraction A) and in 770 to 950 ml (Fraction B) of effluent weighed 14 and 11 mg, respectively. A mass spectrum of the methyl ester of Fraction B was practically identical with that of PGE₁ methyl ester.

Fractions A and B were subjected to paper chromatography with prostaglandin E₁ (PGE₁), PGF₁, and PGF₂ as standards, with the use of ethylene chloride-heptane (50:50) as moving phase, and acetic acid-water (70:30) as stationary phase. The chromatograms were developed for 10 hours by the descending technique (11).

The chromatographic behavior of Fraction B in this system is

\[
\text{HOOC}\left(CH_2\right)_{14}CHCHCHCH=CH(OH)-(CH_2)CH_2
\]

\[
\text{O=C HCO \quad CH}_2
\]

\[
\text{PGF}
\]

\[
\text{HOOC}\left(CH_2\right)_{14}CHCHCHCH=CH(OH)-(CH_2)CH_2
\]

\[
\text{O=C HCO \quad CH}_2
\]

\[
\text{PGF}_{1-1} \text{ and } \text{PGF}_{2-1}
\]

Fig. 1

Fig. 2. Chromatogram of material present in aqueous ethanol extract, showing Fractions A and B. For further information, see the text.

1 The abbreviations used are: PGE, prostaglandin E; PGF, prostaglandin F.
and the solution was acidified with ture followed by 1 hour at room temperature. Water was added, combined ether phases were washed with water until neutral re-

reaction mixture was kept for 15 minutes at ice-bath tempera-

added 5 mg of tritium-labeled sodium borohydride (12). The tritium-labeled sodium borohydride to labeled PGFl and PGF2, of which the former is identical with PGF, isolated pre-

viously from sheep vesicular glands. Thus, if Fraction B were PGF2, of which the former is identical with PGF, isolated pre-

278 rnp (4).

paper (14) that the keto group in PGE can be reduced by sodium 

of Fractions A and B with alkali gave an absorption band at 

violet absorption after alkali and acid treatment. Treatment 

was shown that the physiological activity could be fully ac-

identical with that of PGE, whereas Fraction A is slightly more 
polar, appearing between PGE and PGF1. By eluting the material present in corresponding areas of unsprayed papers, it was shown that the physiological activity could be fully ac-

for in these zones. These eluates were also used to provide material of higher purity for determination of ultra-

C-H bond (4).

This method has been illustrated by synthesizing p-nitrophenyl 
diazoacetate, and allowing it to acylate chymotrypsin. The resulting diazocetylchymotrypsin was then photolyzed in aque-

Recently, several groups of investigators (1-3) have reported the use of multifunctional reagents to help map the regions around the active sites of enzymes. This communication re-

including involving an aliphatic diazo group into a reagent, which can then react with the enzyme at its active center. The diazo group is thus held by chemical bonding near the active site; subsequently, this group can be converted by photolysis, under mild conditions of temperature and pH, to a reactive 
carbene. The carbene will then react irreversibly with the surrounding molecules, even being capable of insertion into a 
c- II bond (4).

R-O- e hv -CHNz - R-O- e -CH: + Nz

O

O

R-O- C-CH2 A

This method has been illustrated by synthesizing p-nitrophenyl diazoacetate, and allowing it to acylate chymotrypsin. The resulting diazocetylchymotrypsin was then photolyzed in aque-

The ester was prepared as follows. Carbobenzoxyglycine was 

of dicyclohexylcarbodiimide at 0°. The resulting p-nitrophenyl 
carbobenzoxyglycinate, after recrystallization from dilute etha-

of 6°. The resulting p-nitrophenyl carboxbenzoxylvinenate, after recrystallization from dilute ether-

ester was then converted to p-nitrophenyl glycinate hydrobromide (6): 2.8 g of the latter in 6 ml of water and 16 ml of methylene 

chloride were diazotized under nitrogen with 1.5 g of sodium 
nitrite in 5 ml of water by the addition of 3 ml of 5% sulfuric 

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