Origin of prostaglandins in human semen

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Summary. The prostaglandins, PGE-1, PGE-2, PGF-1 α , PGF-2 α , 19-OH-PGE-(1 + 2), 19-OH-PGF- α were estimated by RIA in the semen of (a) normal men of proven fertility (total semen or split ejaculate); (b) men 1 year after vasectomy; and (c) men with agenesis of the vas deferens and seminal vesicles. PG concentrations in the semen of vasectomized men were largely within the normal ranges. In men with agenesis of the vas deferens and seminal vesicles, PG concentrations were 10^2 – 10^5 -fold less than that measured in normal subjects. The patterns of 19-OH-PGs and PGF- α in the five fractions of a split ejaculate followed in general the fructose profile. These data suggest that prostaglandins mostly originate in the seminal vesicles.

Introduction

Although it is generally believed that prostaglandins (PGs) present in human semen are secreted by the seminal vesicles, as has been shown in the ram, the experimental proof has not yet been obtained, not even for PGE-2 and PGF-2 α , which have been identified for some time (Eliasson, 1959; Cenedella, 1975; Fuchs & Chantharaksri, 1976). There is, in fact, only limited direct evidence for the synthesis of human seminal PGs by the seminal vesicles (Eliasson, 1959; Bygdeman & Eliasson, 1969; Hamberg, 1976). Moreover, the 19-hydroxylated PGs, and not the prostanoid derivatives studied previously (PGE, PGF or 19-OH-PGA, PGB), are the major prostaglandins in the human semen (Taylor & Kelly, 1974; Templeton, Cooper & Kelly, 1978). A correlation between fructose and 19-hydroxy-PGE levels in human seminal plasma suggests that these PGs are secreted by the seminal vesicles (Conte, Laguzzi, Gioveno, Dondero & Isidori, 1979). The present study was undertaken to attempt clarification of the origin of prostaglandins in human semen.

Materials and Methods

Materials

All unlabelled PGs were a gift from Dr J. Pike (UpJohn Company, Kalamazoo, Michigan, U.S.A.). Tritium-labelled PGs were used for the PGE-1, PGE-2, PGF-1 α and PGF-2 α assays. The iodinated tracers for 19-OH-PGE-2, 19-OH-PGF- α , and the antisera for all the PG radioimmunoassays, were prepared as previously described (Maclouf, Pradel, Pradelles & Dray, 1976; Gerozissis & Dray, 1981).

The semen of selected groups of men was analysed.

Men with proven fertility. The fertility of these men was checked by two criteria, i.e. paternity within the past 10 years and normal spermiogram values (ejaculate volume 2–6 ml, $>20 \times 10^6$ spermatozoa/ml, sperm motility $\geq 50\%$). Samples with normal spermiogram values were also used from students without children; these ejaculates were collected in 5 fractions (Eliasson & Lindholmer, 1976).

Vasectomized men. These men had children <10 years of age and normal spermiogram values before operation. The semen was collected 1 year after vasectomy and was free from spermatozoa.

Men with an obstructive sterility (ejaculatory duct and seminal vesicle agenesis). The lack of patency of the vas deferens was suggested by an azoospermia accompanied by an absence of seminal fructose and an elevated acid phosphatase; it was confirmed by clinical examination and surgery. All the men were asked to abstain from coitus for 3 days before collection of the samples by masturbation within 5 min of ejaculation. Part (0.1 ml) of each sample was placed in liquid nitrogen and stored until assayed for prostaglandins. Another part of the semen samples was allowed to stand for 30 min after collection at room temperature (20–25°C) for liquefaction and then tested for quality (volume, number of spermatozoa, sperm motility).

Assays

The prostaglandins were measured by radioimmunoassay. The samples were diluted to 1/10 with 0.1 M-potassium phosphate buffer, pH 7.4, containing 0.02% aspirin, 0.1% glucose and 0.9% (w/v) NaCl, acidified to pH 4 and extracted twice with ethyl acetate. After extraction, the prostaglandins were purified by silicic acid column chromatography (Gerozissis & Dray, 1977, 1981).

The assay methods and the binding characteristics of the antisera to prostaglandins, were as reported previously (Dray, Charbonnel & Maclouf, 1975; Dray *et al.*, 1980; Gerozissis & Dray, 1981). The assays of each of the substances measured were validated by the following tests; non-specific binding (blank, $90 \pm 10\%$), recovery of known amounts of each PG ($>80\%$); the coefficient of variation between and within assays (6–10%). The non-specific binding and the recovery of tritiated PGE-2, PGF-2 α (≈ 1500 d.p.m. added before acidification) were checked for all dosages. The sensitivities of the assays (quantity of PG in pg to give 50% displacement of B_0) were 5 for PGE-2, 10 for PGF-2 α , 15 for PGF-1 α and 19-OH-PGE(1 + 2), 32 for PGE-1 and 150 for 19-OH-PGF- α .

L(–)-Carnitine and fructose determinations, after thawing of samples, took place in 3 steps, i.e. 20 min at -20°C , 30 min at $+4^\circ\text{C}$ and then exposure to room temperature. The separation of spermatozoa and seminal plasma as well as its deproteinization were achieved simultaneously by centrifugation of semen in a molecular filtration cone at 4°C for 20 min. Free L(–)-carnitine determinations on the filtrate were made by a spectrophotometric method (Marquis & Fritz, 1964; Soufir, Marson & Jouannet, 1981).

Fructose determinations were carried out on the filtrate using a specific enzymic method (Bernt & Bergmeyer, 1974).

Results

The PG concentrations in the semen of the various types of men are shown in Table 1. Values in normal and vasectomized men were similar except for that of 19-OH-PGE(1 + 2) in vasectomized men which was significantly lower ($P < 0.05$, Student's *t* test). The PG values in men with obstructive sterility were 10^2 – 10^5 lower than normal values.

More detailed examination was carried out on 4 ejaculates split into 5 fractions (Table 2). A close relation existed between the pattern of 19-OH-PGE(1 + 2), 19-OH-PGF- α and PGF- α (1 + 2)

Table 1. Prostaglandin concentrations ($\mu\text{g/ml}$) in human semen

Prostaglandins	Normal men		Vasectomized men		Men with agenesis of vas	
	No. of men	Mean $\pm$ s.e.m. (range)	No. of men	Mean $\pm$ s.e.m. (range)	No. of men	Range $\times 10^{-3}$
19-OH-PGE-(1 + 2)	11	375 $\pm$ 140 (141–640)	8	215 $\pm$ 116 (66–446)	4	9–332
19-OH-PGF- α	9	21 $\pm$ 10 (3–29)	7	16 $\pm$ 6 (7–29)	4	1–116
PGE-2	11	14 $\pm$ 11 (1–34)	8	11 $\pm$ 6 (2–51)	4	0.6–15
PGE-1	11	7 $\pm$ 6 (1–16)	8	9 $\pm$ 16 (0.4–50)	4	0.1–9
PGF-2 α	11	2 $\pm$ 2 (0.3 $\pm$ 7.7)	8	1.3 $\pm$ 1.3 (0.1–4.5)	4	0.2–37
PGF-1 α	11	1 $\pm$ 0.7 (0.1–2.1)	8	0.8 $\pm$ 0.9 (0.1–3.3)	4	0.2–20

Table 2. Prostaglandin (4 cases), fructose and carnitine (3 cases) concentrations in 5 fractions of the split ejaculate of normal men (mean $\pm$ s.e.m., $\mu\text{g/ml}$)

Fraction	19-OH-PGE-(1 + 2)	19-OH-PGF- α	PGE-(1 + 2)	PGF- α (1 + 2)	Fructose	Carnitine
1	139 $\pm$ 52	13 $\pm$ 6	29 $\pm$ 13	3.5 $\pm$ 2	690 $\pm$ 390	5190 $\pm$ 1870
2	210 $\pm$ 72	25 $\pm$ 9	34 $\pm$ 10	5.0 $\pm$ 3	1500 $\pm$ 930	3160 $\pm$ 2090
3	277 $\pm$ 72	34 $\pm$ 9	39 $\pm$ 22	7.0 $\pm$ 4	1870 $\pm$ 1410	1450 $\pm$ 860
4	298 $\pm$ 91	38 $\pm$ 12	40 $\pm$ 21	8.0 $\pm$ 5	2350 $\pm$ 1550	1590 $\pm$ 1470
5	347 $\pm$ 107	44 $\pm$ 12	38 $\pm$ 17	8.0 $\pm$ 4	3010 $\pm$ 1640	680 $\pm$ 600

and fructose, although the PGs appeared earlier in the ejaculate. L-Carnitine concentrations decreased as PG and fructose concentrations increased.

Discussion

The secretions in the semen of men with vas and seminal vesicle agenesis are of prostatic origin. The very low PG content in the semen of such men excludes a prostatic origin of seminal prostaglandins.

After vasectomy, semen is free from spermatozoa and epididymo-testicular secretions; it consists of products which originate in the seminal vesicles, the prostate and the part of vas that includes the ampulla. High PG concentrations in the semen of such men show that the PGs do not originate at the proximal part of the vas, the testis or the epididymis, and that the spermatozoa are not the source of seminal PGs. Spermatozoa are not capable of transforming labelled arachidonic acid (Schlegel, Rotermund, Farber & Nieschlag, 1981). In the present study, in a split ejaculate, carnitine concentrations decrease as PG and fructose level increase. Carnitine originates predominantly in the epididymis (Soufir *et al.*, 1981).

It is therefore suggested that the seminal vesicles are the source of the largest PG fraction in human semen, as shown by the parallelism between the PG and the fructose pattern. These results confirm previous hypotheses and observations (Eliasson, 1959; Conte *et al.*, 1979).

The production of a small part of the seminal PGs, especially PGE-2, at another site (e.g. vas deferens ampulla for instance), cannot be excluded.

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