Radioimmunoassay of prostaglandins in the semen of fertile men

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Summary. Specific antisera and iodinated tracers, as well as strict conditions of sample storage, PG extraction and purification, were used to estimate prostaglandins (µg/ml) in the semen of fertile men: 19-OH-PGE (1 + 2) (350), 19-OH-PGF-α (22), PGE-2 (15), PGE-1 (6·2), PGF-2α (2·3), PGF-1α (1·3), 6-keto-PGF-1α (<1), PGD-2, 13,14-dihydro-15-keto-PGFα and TXB-2 (<0·1). Only PGE-2 (50 pg/ml) was detectable in rat seminal fluid.

Introduction

The reports that the 19-hydroxy-prostaglandins E and F represent most of the prostaglandins (PGs) in the semen of fertile men (Taylor & Kelly, 1974; Jonsson, Middleditch & Desiderio, 1975; Templeton, Cooper & Kelly, 1978) have necessitated the re-determination and re-assessment of the complete PG profile in human semen. The conditions of storage, isolation and sample analysis used by earlier workers probably account for their failure to identify the 19-hydroxy-PGs (Taylor & Kelly, 1974; Jonsson et al., 1975).

We have therefore developed a radioimmunoassay (RIA) for 19-hydroxylated PGs and used iodinated tracers, strict control and standardization of sample storage and extraction conditions. A series of specific RIAs has been used to examine prostaglandins in the semen of fertile men, and in seminal fluid of adult Wistar rats.

Materials and Methods

Materials

All unlabelled PGs were a gift of the Upjohn Company. Tritium-labelled PG was used for PGE-2, PGE-1, PGF-2α and PGF-1α assays. The iodinated tracers for 19-OH-PGE-2, 19-OH-PGF-2α, 6-keto-PGF-1α (the major stable prostacyclin metabolite), PGD-2, thromboxane (TXB-2) and 13,14-dihydro-15-keto-PGF-2α were prepared in this laboratory as previously described (Maclouf, Pradel, Pradelles & Dray, 1976).

Adult Wistar rats were killed with chloroform; fluid was collected from the seminal vesicles and placed immediately in liquid nitrogen. The human semen was from men 30–50 years of age and of proven fertility (with children 3–5 years of age). The samples were supplied by Dr P. Jouannet (Laboratoire d’Histologie et d’Embryologie Cytogénétique, C.H.U. de Bicêtre, France, Service du Pr. David) and all had normal spermogram values. The men had been asked to abstain from coitus for 3 days before collection of the samples by masturbation. Within 5 min
of ejaculation 0.1 ml of each sample was placed in liquid nitrogen and stored until assayed (within 3–4 h). For assay, the samples were diluted to 1/10 with 0.1 M-potassium phosphate buffer, pH 7.4, containing 0.02% aspirin, 0.01% glucose and 0.9% (w/v) NaCl, acidified to pH 4 and extracted twice with ethyl acetate. When a known amount of 19-OH-PGE-2 or 19-OH-PGF-2α was added to a sample of rat seminal fluid and the samples acidified to pH 3 (a pH currently used for primary PGs), instead of pH 4, and extracted with cyclohexane-ethyl acetate, the recovery of 19-hydroxy compounds was <20%. After extraction, the prostaglandins were purified by silicic acid column chromatography. The purification steps have been previously described (Gerozissis & Dray, 1977). The 19-hydroxylated PGs were eluted in the third fraction and thus separated from PGA, PGB, PGE-2 and 13,14-dihydro-15-keto-PGs.

Assay

The assay methods and the binding characteristics of the antisera to 19-OH-PGE-(1 + 2), PGE-1, PGE-2, PGF-1α, PGF-2α, 13,14-dihydro-15-keto-PGF-1α, TBX-2 and 6-keto-PGF-1α were as reported previously (Dray, Charbonnel & Maclouf, 1975; Gerozissis & Dray, 1977, 1981; Sors, Maclouf, Pradelles & Dray, 1977; Sors et al., 1978; Dray, Pradelles, Maclouf, Sors & Bringuier, 1978). The cross-reactivity of the 19-OH-PGE-(1 + 2) antiserum was ≤15% for 19-OH-PGB-2 and <1% for 19-OH-PGA-2. The PGD-2 antiserum used showed a 40% binding for a final dilution of 1/12 000. A 50% decrease of bound radioactivity was obtained with 20 pg PGD-2. Cross-reactivity was <0.1% against PGE-2, PGF-2α and 6-keto-PGF-1α, and 0.3% against TXB-2 (unpublished results).

The antiserum to 19-OH-PGF-2α was raised in rabbits by using 19-OH-PGF-2α coupled to bovine serum albumin (BSA). This antiserum showed 40% binding (B/T × 100) at a final dilution 1/150 000. A 50% increase in bound radioactivity was obtained with 280 pg 19-OH-PGF-2α. Cross-reactivity against PGF-2α, PGF-1α and 19-OH-PGA-2 was 2, 4 and ≤15% respectively and was <1% for 19-OH-PGE-2, PGD-2 and 19-OH-PGB-2. Immuno-reactivity was tested with an iodinated (125I) tracer, 19-OH-PGF-2α coupled to tyramine and purified by t.l.c. The iodinated tracer was prepared in our laboratory as described by Maclouf et al. (1976).

The assays for each of the substances measured were validated by the following tests: non-specific binding (blank, 90 ± 10%); recovery of known amounts of each PG added to rat seminal fluid (>80%); the coefficient of variation between and within assays (6–10%). The non-specific binding and the recovery of tritiated PGE-2, PGF-2α (≈1500 d.p.m. added before acidification) were checked for all dosages. The sensitivities of the assays (pg/ml) were 150 for 19-OH-PGF, 15 for 19-OH-PGF-(1 + 2) and 5–10 for the other substances.

Results

Human semen

The PG concentrations in the semen of fertile men are shown in Table 1. Table 2 shows the degradation of some of the PGs when the samples were not immediately stored in liquid nitrogen. Table 3 shows the PG concentrations in the semen of the same man, collected at different periods.

Table 1. Mean ± s.e.m. concentrations (µg/ml) of prostaglandins in the semen of 14 fertile men

<table>
<thead>
<tr>
<th></th>
<th>19-OH-PGE-(1+2)</th>
<th>19-OH-PGF-α</th>
<th>PGE-2</th>
<th>PGE-1</th>
<th>PGF-2α</th>
<th>PGF-1α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± s.e.m.</td>
<td>350 ± 139</td>
<td>22 ± 9</td>
<td>15 ± 12</td>
<td>6.2 ± 5</td>
<td>2.3 ± 2</td>
<td>1.3 ± 1</td>
</tr>
</tbody>
</table>

The concentration of 6-keto-PGF-1α was <1 µg/ml.
Values of PGD-2, 13,14-dihydro-15-keto-PGF-α(1+2) and TXB-2 were <0.1 µg/ml.
Table 2. Effect of storage conditions on PG concentrations (expressed as %) in human semen

<table>
<thead>
<tr>
<th>Storage conditions</th>
<th>19-OH-PGE-(1+2)</th>
<th>PGE-2</th>
<th>PGE-1</th>
<th>PGF-2α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immediately frozen in liquid N₂</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Frozen after 40 min at 37°C</td>
<td>75</td>
<td>65</td>
<td>70</td>
<td>89</td>
</tr>
<tr>
<td>Frozen after 72 h at 37°C</td>
<td>49</td>
<td>&lt;1</td>
<td>&lt;0.1</td>
<td>71</td>
</tr>
</tbody>
</table>

Table 3. Comparison of PG concentrations (μg/ml) in the semen of the same subject collected at different times

<table>
<thead>
<tr>
<th>Date of collection</th>
<th>19-OH-PGE-(1+2)</th>
<th>PGE-2</th>
<th>PGE-1</th>
<th>PGF-2α</th>
<th>PGF-1α</th>
</tr>
</thead>
<tbody>
<tr>
<td>17 May 1978</td>
<td>415</td>
<td>9.3</td>
<td>3.7</td>
<td>0.6</td>
<td>0.7</td>
</tr>
<tr>
<td>15 June 1978</td>
<td>401</td>
<td>15.0</td>
<td>3.0</td>
<td>1.1</td>
<td>1.1</td>
</tr>
<tr>
<td>10 November 1979</td>
<td>300</td>
<td>25.0</td>
<td>3.7</td>
<td>1.8</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Rat seminal vesicle fluid

In the seminal fluid of adult rats the PGE-2 concentration was 50 pg/ml but 19-OH-PGE-(1 + 2), 19-OH-PGF-α, PGE-1, PGF-1α, PGF-2α, 13,14-dihydro-15-keto-PGF-α(1 + 2) were undetectable (<150 pg 19-OH-PGF/ml; <15 pg 19-OH-PGE-(1 + 2)/ml and <5–10 pg/ml for the other substances. Each estimate was for a pool of about 1 ml fluid taken from the seminal vesicles of 3 rats, and the experiment was repeated three times).

When the seminal vesicles remained for 20 min at room temperature, after dissection, prostaglandins were liberated in vitro: the PGE-2 concentration was 30-fold higher (1500 pg/ml) and concentrations of PGF-2α and 13,14-dihydro-15-keto-PGF-α (1 + 2) were 1300 and 2800 pg/ml respectively.

Discussion

This is the first time to our knowledge that RIA has been used for measurement of 19-OH-PGE and 19-OH-PGF. The values reported here are similar to those measured in human semen by gas–liquid chromatography and mass spectrometry (Templeton et al., 1978).

The difficulty of valid PG measurement in biological fluids unless strict conditions for handling, storage and extraction are respected, is once more emphasized by the results showing degradation of PGs in human semen (Table 2) and in-vitro synthesis of PGs in the rat seminal vesicle fluid.

19-OH-PGE-(1 + 2) represented the major part of the PGs examined, corresponding to 80–90% of the total PGs present in the semen of fertile men, followed by 19-OH-PGF-α and PGE-(1 + 2) (5–10%); PGD-2, TXB-2 and one of the principal PGF metabolites, 13,14-dihydro-15-keto-PGF-α (1 + 2) represented an insignificant fraction (<0.2%) of the total PGs. 6-Keto-PGF-1α has been identified in several organs and tissues (Sun, Chapman & McGuire, 1977). We found very low concentrations in the semen and the presence of prostacyclin in this fluid is therefore probably unimportant.

Only PGE-2 (50 pg/ml) was present in measurable amounts in the seminal fluid of adult rats. Together with previous results, the present findings indicate the need for re-examination of the possible relationship between seminal PG concentration and human sterility (Kelly, 1978).
is no convincing explanation for such large concentrations of PGs in the semen. A physiological role in the metabolism of spermatozoa or an effect upon the female reproductive tract have been suggested and PGEs and 19-OH-PGE-1 have been shown to interfere with uterine contraction (Martin & Bygdeman, 1975; Russell, Taylor & Kelly, 1979). In addition, PGE-1 and 19-OH-PGEs cause an increase in glucoxylysis and a fall in CO₂ liberation by washed, ejaculated human spermatozoa (Kelly, 1978).

References


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