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Determination of Urinary Prostaglandin E₂ by the Use of Prostaglandin F_{2α} Antiserum and Sodium Borohydride

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A radioimmunoassay (RIA) for prostaglandin E₂ (PGE₂) based on the use of commercial PGF_{2α} antiserum is described. PGE was extracted from human urine and separated by silicic acid column chromatography, then PGE was converted to PGF by the use of sodium borohydride (NaBH₄), and the formed PGF_{2α} was determined radioimmunologically with commercial PGF_{2α} antiserum.

There was a good correlation ($y=0.841x+0.518$, $r=0.912$) between added standard PGE₂ and measured PGE₂ levels in human urine. Moreover, a good correlation ($y=1.46x+0.569$, $r=0.952$) was also found between the PGE₂ levels in the urine obtained by means of the bioassay previously reported and those obtained by the proposed method.

Therefore, this method has high accuracy and good reproducibility. Furthermore, it is also an advantage that radioimmunological assays of PGF_{2α} and PGE₂ in biological samples can be performed separately with only one antiserum by this method.

Keywords—determination on prostaglandin E₂; prostaglandin F_{2α} antiserum; radioimmunoassay; urinary prostaglandin E₂; sodium borohydride

It has been reported that the level of urinary prostaglandin (PG) reflects the production and/or release of PG in the kidney.¹⁾ Moreover, it is known that in general, PG excreted in urine is mainly PGE₂.²⁾

We have previously reported on the assay of PGE and F by making use of the contractions of rat stomach fundus or uterine strips,³⁾ and the levels of PGE in kidney, urine, and ovary have been determined by the application of this method.⁴⁾ Although this method has the advantage that it is based on the biological activity and has good reproducibility, it is difficult to determine a large number of samples at once. Radioimmunoassay (RIA) is advantageous from this point of view. At present, commercially obtainable PGB₁ antiserum (Clinical Assays) is applied to the determination of PGE. However, this assay is performed with PGB₁ antiserum after conversion of PGE₁ to PGB₁ by alkaline treatment, so that it is difficult to apply this assay to determine PGE in biological samples rich in PGE₂ such as urine, since PGE₂ is converted to PGB₂ by alkali. On the other hand, the determination of PGF_{2α} using commercial PGF_{2α} antiserum (Clinical Assays) shows fairly good specificity and reproducibility. Therefore, the present study describes a procedure for PGE₂ determination by making use of PGF_{2α} antiserum after conversion of PGE to PGF with sodium borohydride (NaBH₄), a specific reducing agent for ketones.

Experimental

Exp. 1—Eight ml of normal male human (age = 29) urine was collected at 10:00 a.m. Standard PGE₂ (Upjohn) was added at 0, 0.5, 1, and 2 ng per 2 ml of the urine. Then the urine was acidified to pH 3 with 1 N hydrochloric acid, and PGs in the urine were extracted with 6 ml of ether. The ether layer was evaporated to dryness at 37 °C under an atmosphere of N₂ gas. The extracted PGs were dissolved in 0.5 ml of benzen-ethyl acetate-methanol (60:40:10), and the solution was applied to a silicic acid column for separation of PGE as reported previously.⁵⁾ The PGE

fraction was collected, and the PGE extract was dissolved in 0.5 ml of ethanol. The reducing agent, NaBH_4 , was dissolved to give a 6% solution in 70% ethanol. Ten μl of the NaBH_4 solution was added to the ethanol solution, and the mixture was transferred to an ice water bath for 10 min, then kept at room temperature for 20 min.⁶⁾ The reacted solution was dried under N_2 gas, and the residue was dissolved in a definite volume of ethanol. An aliquot of the ethanol solution was removed to a test tube for RIA and dried. The PGF formed was measured radioimmunologically with the commercial $\text{PGF}_{2\alpha}$ antiserum. It is known that PGE_2 , on treatment with NaBH_4 , is converted to a mixture of $\text{PGF}_{2\alpha}$ and $\text{PGF}_{2\beta}$.⁷⁾ Therefore, a definite amount of standard PGE_2 was simultaneously reduced, and the serologically active product was assayed with $\text{PGF}_{2\alpha}$ antiserum in order to prepare a standard curve.

Exp. 2—The normal male human (age=22–39) urine of 10 persons was collected between 9:30 and 10:30 a.m. Urinary PGE was extracted and separated as described above. The PGE fraction was divided into two portions. One was determined by RIA in the same way as in Exp. 1, and the other by bioassay according to the method described in the previous report.^{3a)}

Results and Discussion

Exp. 1 was designed to examine the accuracy of the measurement, and the results are shown in Fig. 1. A good correlation ($y=0.841x+0.518$, $r=0.912$) was recognized between the amounts of PGE_2 and the measured PGE_2 values. The data indicated a mean coefficient of variation of 10.9%. Zero ng of standard PGE_2 added to urine gave a value of 0.5 ng. This means that the urine itself contained 0.5 ng of PGE_2 per ml.

The PGE_2 levels in normal male human urine were determined by both RIA and bioassay. As shown in Fig. 2, a good correlation ($y=1.46x+0.569$, $r=0.952$) was found between the two assays. Urinary PGE_2 concentration revealed individual variations, and the values obtained by RIA did not differ largely from those found by bioassay. The values obtained by RIA were always higher than those by bioassay. The explanation for this result remains obscure at present; however, it can be presumed that some substances in urine might interfere with the binding of PG to antiserum or with the contraction of smooth muscle induced by PG.

The cross reactivities of the $\text{PGF}_{2\alpha}$ antiserum were 28.2% for $\text{PGF}_{1\alpha}$, 1.2% for 13,14-dihydro-15-keto $\text{PGF}_{2\alpha}$, and less than 0.2% for PGA_1 , PGA_2 , PGE_1 , and PGE_2 .

Consequently, this method of measuring urinary PGE_2 by using $\text{PGF}_{2\alpha}$ antiserum after the conversion of PGE to PGF with NaBH_4 has high accuracy and good reproducibility. Furthermore, it also has the advantage that the radioimmunological assays of $\text{PGF}_{2\alpha}$ and PGE_2 can be performed separately with only one antiserum by the proposed method. Thus,

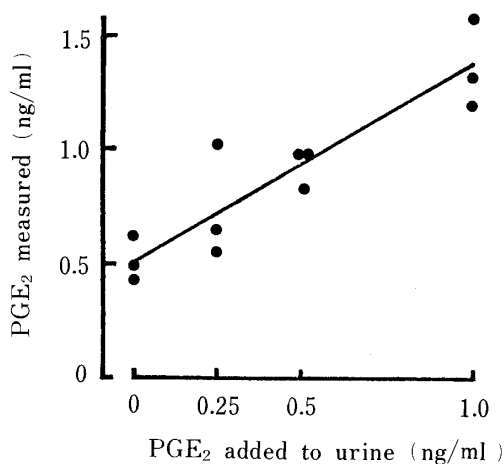


Fig. 1. Recovery of PGE_2 Added to Normal Male Human Urine
 $y=0.841x+0.518$
 $r=0.912$.

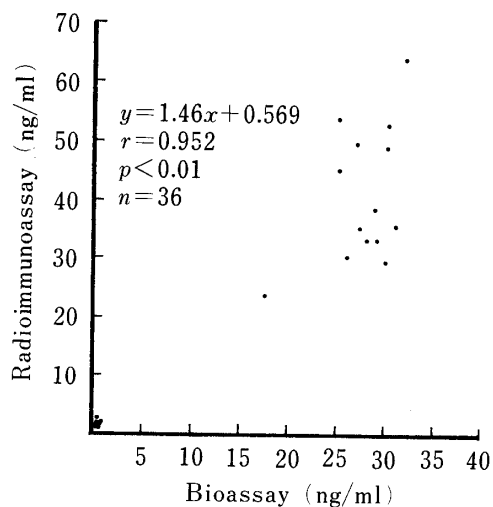


Fig. 2. Prostaglandin E_2 Levels in Normal Male Human Urine as Determined by Bioassay and Radioimmunoassay

the method should be useful for determination of PGE in biological samples, especially PGE₂-rich samples such as urine.

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