Sex and age differences in the specific activity of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase in human fetal kidney tissue

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Summary. The specific activity of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) was significantly greater ($P < 0.005$) in human kidney tissue obtained from male abortuses aged 10–16 weeks after conception than in female abortuses of the same gestational age and in younger male and female abortuses. There was a significant correlation ($r = 0.93$, $P < 0.001$) between the specific activity of PGDH in fetal kidney and testicular tissues obtained from human abortuses of 10–26 weeks gestational age. These results suggest a role for testosterone in the regulation of PGDH activity in the fetal kidney.

Introduction

During a study of the regulation of the concentrations of prostaglandins in human fetal organs, the specific activity of NAD⁺-dependent 15-hydroxyprostaglandin dehydrogenase (PGDH) in various fetal tissues was determined. Except for the adrenal gland and the chorion laeve, the highest specific activities of the enzyme were found in kidney and associated structures, e.g. the ureter. The mean specific activity of PGDH in kidney tissue obtained from human abortuses aged 10–16 weeks after conception was greater than that in kidney tissue of abortuses before this age. However, the specific activity of PGDH was not high in kidney tissues of all abortuses at this gestational age. Since 10–16 weeks gestation is the time when testosterone levels attain maximum levels in human male fetuses (Abramovich & Rowe, 1973; D'Aux & Murphy, 1974; Reyes, Winter & Faiman, 1974), we examined whether the difference in specific activity of PGDH in kidney tissues from various abortuses was related to the sex of the fetus.

Materials and Methods

Tissues

Tissues were obtained from human abortuses 6–26 weeks after conception in accordance with the Donors Anatomical Gift Act of the State of Texas and after obtaining written consent from the women to be aborted employing a consent form and protocol approved by the Human Research Review Committee of the University of Texas Health Science Center at Dallas, Texas. Most of the abortions were performed by dilatation and extraction but a few were accomplished by abdominal hysterectomy. Gestational age was estimated from fetal foot length (Streeter, 1920; Moore, 1973). The tissues were transferred immediately to a solution of ice-cold 0.15 M-NaCl. The capsule of the kidney and contiguous adventitious tissues were removed and discarded, as were the major calyces of the kidney.

Assay of NAD⁺-dependent PGDH activity

The kidney and testicular tissues were homogenized (Potter-Elvehjem tissue grinder) in 10 volumes of ice-cold Tris-HCl (50 mM, pH 7.2) buffer containing glycerol (20%, v/v), sucrose (250 mM), EDTA (1 mM), and dithiothreitol (2 mM). The supernatant fraction obtained following centrifugation at 105 000 g for 1 h at 2°C was assayed for PGDH activity by a spectrofluorometric method previously described in detail (Casey & Johnston, 1980). Briefly, assays were conducted in sodium carbonate-bicarbonate buffer (50 mM, pH 9.5) containing NAD⁺ (1 mM), prostaglandin (PG) E-2 (70 µM, added in 5 µl of ethanol), and cytosolic protein (5 to 50 µg) at 25°C. The final volume of the reaction mixture was 1 ml. The rate of increase in fluorescence due to NADH formation was monitored at an excitation wavelength of 340 nm and an emission wavelength of 442 nm. The specific activity of PGDH was computed following correction for (1) endogenous cytosolic enzymic activities leading to NADH formation in the absence of added PGE-2 and (2) a non-enzymic reaction which takes place at pH 9.5 in the presence of NAD⁺ and PGE-2 but in the absence of tissue.

Determination of the sex of the fetus

Since most tissues were obtained from specimens aborted by dilatation and extraction, the sex of the fetus ordinarily could not be ascertained by physical examination and the method of quinacrine fluorescence of the Y chromosome (Korn, Gauden, Baxter & Herndon, 1978) with modifications was employed. Most commonly, fetal cells were obtained from the mucosal lining of the small intestine, but in some instances from the mucosal lining of the stomach, from fetal skin, or from fetal adrenal tissue. In each case the cells were dispersed by mechanical agitation of the tissue and transferred to centrifuge tubes in 0.15 M-NaCl. The suspension was centrifuged at 300 g for 10 min. The supernatant solution was removed and discarded. The cells were fixed in a solution of methanol:acetic acid (3:1, v/v) for no less than 20 min. Aliquots of the dispersed cells were transferred to microscope slides and dried in air. The slides, with cells affixed, were placed in a solution of citrate-phosphate buffer (McIlvaine, 1921), pH 4.2, for 5 min, and then transferred to a solution of 0.5% (w/v) quinacrine dihydrochloride in citrate-phosphate buffer, pH 4.2, for 5 min. After quinacrine treatment, the slides were rinsed with distilled water and placed in a solution of citrate-phosphate buffer, pH 4.2, for 5 min. Thereafter, the slides were rinsed with distilled water, blotted dry, and a wet mount was prepared in the citrate-phosphate buffer. The nuclei of the cells were examined for fluorescence of the Y chromosome by using a halogen lamp as the light source and an FITC excitation filter. In 30 abortuses in which the sex was known on the basis of physical criteria or on the grounds of identification of testes or uterine structures, sex was ascertained correctly in every case employing the quinacrine fluorescence technique.

Protein measurement

Cytosolic protein was assayed as described previously (Casey & Johnston, 1980) by a modification of the method of Lowry, Rosebrough, Farr & Randall (1951); sodium deoxycholate (final concentration, 0.1% w/v) was added as the precipitating carrier and trichloroacetic acid (final concentration, 2.5%, w/v) for precipitation. Bovine serum albumin was used as the standard.

Statistical analysis

The data were analysed by Bartlett's test for equal variances, and when these were equal the significance of differences was assessed by Student's *t* test. When the variances were not equal, the Mann-Whitney test was applied.

Results

As shown in Table 1, the specific activities of PGDH in kidney tissues obtained from female and male abortuses at 6–10 weeks gestational age were similar. However, at 10–16 weeks PGDH activity was significantly greater in male abortuses than in female abortuses of the same age and significantly greater than that in younger male abortuses.

PGDH was assayed in testicular and kidney tissues obtained from 11 abortuses of 10–26 weeks gestational age and a close correlation ($r = 0.93$, $P < 0.001$) was found between the specific activities of this enzyme in these tissues (Text-fig. 1).

Table 1. Mean $\pm$ s.e.m. values for the specific activity of PGDH in human fetal kidney

Gestational age (weeks after conception)	Sex	No. of fetuses	Specific activity of PGDH (nmol.min ⁻¹ .mg protein ⁻¹)
6–10	Female	9	3.90 $\pm$ 0.42
	Male	11	4.04 $\pm$ 0.39
10–16	Female	24	4.26 $\pm$ 0.45
	Male	38	7.34 $\pm$ 0.70*

* Significantly different from value for females of same age ($P < 0.005$) and from males aged 6–10 weeks ($P < 0.02$).

Text-fig. 1. Correlation between specific activity of PGDH in human fetal kidney and testicular tissue ($r = 0.93$, $P < 0.001$).

Discussion

In the human fetus, plasma concentrations of testosterone in the male only transiently are greater than those in the female. During the last half of pregnancy, testosterone concentrations in cord blood of male and of female abortuses and newborns are similar. It has been demonstrated, however, that between 10 and 16 weeks gestational age (computed from the time of fertilization) the testosterone values in umbilical cord blood are greater in male than in female fetuses (Abramovich & Rowe, 1973; D'Aux & Murphy, 1974; Reyes *et al.*, 1974). In the present study, we found that the specific activity of PGDH was significantly greater in kidney tissues obtained from male abortuses than from female abortuses between 10 and 16 weeks gestational age. Gecse, Ottlecz, Schaffer, Bujdose & Telegdy (1979) have reported that the specific activity of PGDH in kidney tissue was greater in male than in female rats. We found that values for 10–16-week-old male abortuses were greater than those for younger male abortuses.

The kidney is known to be responsive to androgen action. Androgen treatment of male rats results in an increase in the specific activity of the microsomal enzyme, 3 α -hydroxysteroid dehydrogenase, in kidney tissue (Ghraf, Lax, Hoff & Schriefers, 1975) and appears to prime the hyperinductive oestrogen effect on the activity of cytosolic 3 α -hydroxysteroid dehydrogenase in the kidney (Ghraf, Lax & Schriefers, 1977).

Based on the results of the present study, it seems reasonable to conclude that androgen, secreted by the human fetal testis, brings about an increase in the specific activity of PGDH in the kidney. The correlation between the specific activities of PGDH in testicular and kidney tissues suggests a role for testosterone in modulating PGDH activity and thence prostaglandin levels in these tissues. It may be that testosterone or its active metabolite, dihydrotestosterone, acts directly on kidney tissue to bring about an increase in the activity of PGDH, or testosterone may act to attenuate the action of oestrogen since oestrogen treatment of rats caused a decrease in the specific activity of PGDH in kidney tissue (Blackwell & Flower, 1976). The present results support the proposition that testosterone may act in fetal tissues other than those involved directly in male differentiation of internal and external genital anlagen, for example the fetal brain. Since fetal kidney tissue can be maintained for several days in organ culture, the human fetal kidney may represent an ideal tissue for the in-vitro study of the molecular events involved in androgen action. PGDH activity may prove to be a marker of androgen action. If this is the case, it will be of considerable interest to evaluate the levels of prostaglandins in urine of persons with the androgen resistance syndrome because urinary prostaglandins are believed to be formed and metabolized primarily in the kidney (Pace-Asciak, 1978).

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