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SEX DIFFERENCE IN STIMULATORY ACTIONS OF COFACTORS ON PROSTAGLANDIN SYNTHETASE IN MICROSOMES FROM RAT KIDNEY MEDULLA

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Abstract—The stimulatory action of cofactors on PG synthetase in the microsomal fraction of rat kidney medulla has been examined in relation to the sex of the animals. Norepinephrine stimulated the activity of PG synthetase to the same degree in males and females. Reduced glutathione caused a much greater increase in the PG synthetase activity in females than in males. In females reduced glutathione was a stronger activator of PG synthetase than norepinephrine, whilst in males it was a poorer stimulator. These results suggest that there may be a sex difference in the PGE isomerase which converts PG endoperoxides to PGE, possibly due to sex hormones.

Prostaglandin (PG) synthetase in the microsomal fraction of a variety of tissues is stimulated by various natural phenolic compounds and reduced glutathione (GSH) [1–6]. GSH is possibly specifically involved as a coenzyme in PG endoperoxide E isomerase [7] and PG endoperoxide D isomerase [8]. These results indicate that the phenolic compounds and GSH may be the endogenous cofactors involved in regulating the biosynthesis of PGs *in vivo*.

When extracts of brain homogenized in Krebs solution were chromatographed for group separation of PGs, in female brains only material running as PGE was detected whereas in male brains PGF-like or PGE+PGF-like material was found [9]. There were also sex differences in the effect of indomethacin on the PG biosynthesis [9], and in the content of PGs in brain [9, 10]. Furthermore, PG synthetase activity in rat kidney medulla microsomes was significantly higher in males than in females [11].

Although only a little is known regarding the endogenous regulating mechanism of the generation of PGs, these data suggest one possibility that the actions of cofactors on PG synthetase may differ between males and females. In this investigation, therefore, it was decided to examine the stimulatory action of cofactors on PG synthetase activity in rat kidney medulla in relation to the sex of the animals.

MATERIALS AND METHODS

Materials. Arachidonic acid (Grade I), GSH and bovine serum albumin were purchased from Sigma Chemical Co., St. Louis, MO. Norepinephrine bitartrate (NE) was the product of Wako Pure Chemical Industries, Ltd., Osaka, Japan. PGE radioimmunoassay kit was obtained from Clinical Assays Inc., Cambridge, MA. Authentic PGB_2 was a gift from Ono Pharmaceutical Co., Ltd., Osaka, Japan.

Assay of PG synthetase. Adult Wistar rats of either sex weighing 250–300 g were decapitated, and the kidneys were rapidly removed. The medulla was dissected out and homogenized in 10 vol. 0.1 M Tris–HCl buffer, pH 8.0, with a Potter–Elvehjem homogenizer. The homogenates were centrifuged at 12,000 *g* for 20 min. The supernatant fractions were diluted and further centrifuged at 105,000 *g* for 60 min in a Beckman model L5-65 ultracentrifuge. The resultant microsomal pellet was suspended by homogenization in 0.1 M Tris–HCl buffer, pH 8.0. This microsomal fraction was used for assay of PG synthetase according to the method of Pong and Levine [5]. A 0.5 ml portion of the fraction containing 0.3–0.4 mg protein was incubated at 37° with arachidonic acid as a substrate. NE and GSH were used as cofactors of PG synthetase and each of them was added to give a final concentration of 1 mM [4]. At termination of the incubation, the reaction mixture was boiled for 1.5 min and diluted to 5.0 ml with ice-cold Tris–isogelatin buffer (0.01 M, pH 7.4, containing 0.14 M NaCl and 0.1% (w/v) gelatin). After removal of the denatured protein by centrifugation, PGE was determined in the supernatant fluid (1.0 ml).

Protein concentration was measured by the method of Lowry *et al.* [12] with bovine serum albumin as the standard.

Determination of PGE_2. PGE_2 was determined by radioimmunoassay using a commercially available PGE radioimmunoassay kit. The kit protocol is a modification of the methods of Levine *et al.* [13] and Gutierrez-Cernosek *et al.* [14]. Briefly, PGE_2 was converted to PGB_2 by alkaline treatment, and which was assayed with anti-PGB_1 rabbit serum. A standard curve was obtained using authentic PGB_2 in the range of 41 pg–10 ng, and only the linear part of the curve (100–4000 pg) was used. PGB_2 required for 50 per cent inhibition of the binding of [^{3}H]PGB_1 to the

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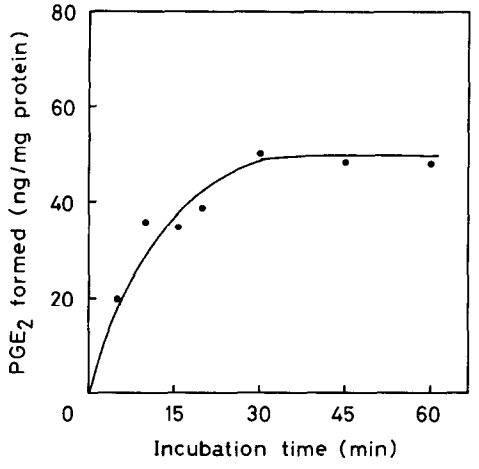

Fig. 1. Effect of incubation time on PG synthesis by the microsomal fraction of kidney medulla of male rats. The fractions were incubated at 37° with 130 μM arachidonic acid for the indicated time in the presence of 1 mM NE and 1 mM GSH. PGE_2 formed was determined by radioimmunoassay. Each point is the mean of duplicate measurements.

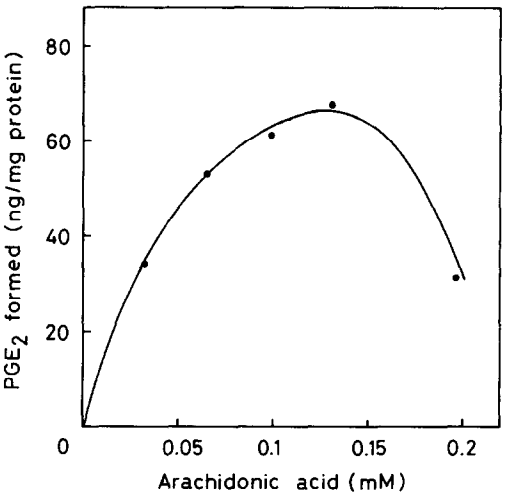

Fig. 2. Effect of substrate concentration on PG synthesis. The fractions were incubated at 37° for 30 min with various concentrations of arachidonic acid. Other details are as given in Fig. 1.

antiserum was 610 pg. The activity of PG synthetase was represented as ng PGE_2 formed/mg protein.

Statistical analysis was performed using Student's *t*-test.

RESULTS

The incubation of the microsomal fraction of rat kidney medulla with arachidonic acid resulted in the formation of PGE_2. As shown in Fig. 1, the PG formation increased during the first 30 min of incubation and then ceased. The effect of arachidonic acid concentration on PG formation is shown in Fig. 2. PG production was increased to a peak at 130 μM arachidonic acid with apparent K_m value 2.4 μM. Therefore, this concentration was used subsequently.

The incubation of the microsomal fraction from either males or females with arachidonic acid in the absence of cofactors produced small but consistent amounts of PGE_2 (Table 1). The tendency for a slightly higher PG production in males is not statistically significant ($P > 0.1$). NE or GSH significantly enhanced the PG production in preparations from both sexes, but although NE alone stimulated the activity of PG synthetase by a similar ratio in both sexes, more PGE_2 was formed by preparations from males than from females. However, GSH enhanced PG formation in preparations from females more than from males. In males GSH stimulated PG synthetase less than NE, whereas in females the reverse occurred.

Simultaneous addition of NE and GSH caused an increase in the PG formation in preparations from both sexes, but the ratio of the increase was greater in females than in males.

DISCUSSION

Inhibition of enzymatic formation of PGE_2 by high concentrations of arachidonic acid has been reported for rabbit kidney medulla microsomes by Schwartz-

Table 1. Sex differences in stimulatory action of NE (1 mM) and GSH (1 mM) on PG synthetase in the microsomal fraction of rat kidney medulla*

Addition	Sex	Number of experiments	PGE_2 formed† (ng/mg protein)	Ratio of increased PGE_2 formed
None	M	6	11.3 ± 1.3	
	F	8	8.4 ± 0.98	
NE	M	5	64.8 ± 5.5	5.9
	F	5	47.8 ± 4.3‡	5.7
GSH	M	7	30.5 ± 4.9	2.8
	F	6	57.0 ± 2.7§	6.8
NE + GSH	M	5	70.2 ± 13.7	6.4
	F	5	63.5 ± 4.4	7.6

* The microsomal fractions were incubated with 130 μM arachidonic acid for 30 min at 37°.

† Values are means ± S.E.

‡ $P < 0.05$, § $P < 0.001$; males vs females.

man *et al.* [15] and for bovine seminal vesicle microsomes by Flower *et al.* [2] and Robak *et al.* [16]. In agreement with these results, our studies showed a pronounced substrate inhibition of PG formation at 0.2 mM arachidonic acid in rat kidney medulla microsomes. The K_m values reported by Schwartzman *et al.* [15], Robak *et al.* [16] and ourselves are about 10, 7.6 and 2.4 μM, respectively.

The activity of PG synthetase in the microsomal fraction of rat kidney medulla was higher in males than in females in the presence of NE alone, whereas the activity was much higher in females in the presence of GSH alone. Similar tendencies either in the absence or in the presence of NE + GSH did not reach statistical significance, although Gecse *et al.* [11] found that the PG synthetase activity in rat kidney medulla microsomal fraction in the presence of NE and GSH is higher in males than in females. However, Gecse *et al.* [11] used the microsomal fraction containing 2–3 mg protein, incubated with 0.1 μM [^{14}C]arachidonic acid in the presence of 1 mM NE and 2 mM GSH, and the radioactive PGs were analysed by thin layer chromatography, whereas in this investigation the microsomal fraction containing 0.3–0.4 mg protein was incubated with 130 μM arachidonic acid, and PGE_2 formed was determined by radioimmunoassay. Thus, since at a low substrate–enzyme ratio the sex difference appears, it seems likely that the effect of the substrate concentration on PG synthetase may differ between males and females, but this would not affect the interpretation of our data for stimulatory actions of cofactors.

The present studies have also demonstrated that there are sex differences in the stimulatory action of cofactors on PG synthetase in the microsomal fraction of rat kidney medulla. Tai *et al.* [4] reported that GSH stimulated kidney medulla PG synthetase less than does epinephrine or NE. This accords with our results in males, but we have found that in females GSH is a stronger stimulator of PG synthetase than NE.

PG synthetase activity in microsomal fraction is stimulated by GSH [1–6], tryptophan [3], epinephrine [2, 4], NE [4] or hydroquinone [5, 17]. Phenolic compounds such as NE or tryptophan stimulate the synthesis of the endoperoxide intermediates and are supposed to be cofactors for cyclooxygenase, a constituent of PG synthetase [3, 18]. GSH facilitates the formation of PGs from endoperoxide [3, 18]. Either cofactor leads to a greater synthesis of all the primary PGs [4]. PG endoperoxide E isomerase, which catalyses the PGE formation from endoperoxide, requires GSH both for the reaction and for enzyme stability [7]. Hence, our results suggest that there may be a sex difference in PG endoperoxide E isomerase.

GSH has also been implicated in the conversion of 15-hydroperoxy-endoperoxide into 15-hydroxy-endoperoxide by the action of glutathione peroxidase [1]. The activity of this enzyme is higher in mature female rats than in mature male rats [19], due to oestradiol and progesterone [20]. These hormones also affect PG synthesis by several tissues *in vivo* [21, 22] and *in vitro* [22–24]. Treatment of gonadectomized rats with sex hormones, particularly oestradiol, affects PG synthesis by kidney medulla microsomes.* Therefore, sex hormones may be responsible for the sex difference in PG formation. Another possibility, which remains to be elucidated, is that altered activity of intracellular glutathione peroxidase in response to sex hormones [20] may subsequently modify PG biosynthesis with respect to the action of cofactors.

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