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Inhibitory Effect of Estradiol-17β on Human Chorionic Gonadotropin-Induced Increment of Testicular Blood Flow and Plasma Testosterone Concentration in Rats

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ABSTRACT

The effects of human chorionic gonadotropin (hCG) and estradiol-17β (E_2) on testicular blood flow and plasma testosterone concentrations were measured in rats. Injection of 100 IU hCG 20–24 h prior to experiment resulted in a significant rise in testicular blood flow and plasma testosterone concentration. Daily treatment with 50 μg E_2 for 5 days did not affect testicular blood flow but induced a significant depression of plasma testosterone concentration. However, E_2 treatment did partly diminish the stimulatory effect of hCG on both testicular blood flow and the concentration of testosterone in plasma. It was concluded that blood flow changes are involved in the effect of hCG on testicular endocrine function and that E_2 can partly inhibit these effects.

INTRODUCTION

The stimulatory effect of LH and hCG on testicular steroidogenesis is well established, whereas the effect on testicular blood flow is less clear. Infusion of LH to anesthetized rats caused a small but significant decrease in the vascular resistance of the testis (Damber and Janson, 1978a). However, other investigators (Nguyen-Duc-Kien, 1975; Free, 1977; Amann et al., 1978) did not find any significant circulatory effects in the testis following gonadotropin injections. The reason for this difference may be that the acute vascular effect of LH is very small and there could also be differences due to the sensitivity of the method used for testicular blood flow measurement. Hartman et al. (1950), using a morphological technique, found a hyperemic effect in the rat testis in response to hCG. Furthermore, Pirke et al. (1979) presented data indicating that the reduced testicular blood flow observed in the aged rat was completely reversible by hCG treatment. It has also been shown that the testicular lymph flow increases 16–20 h after hCG treatment (Sharpe and Setchell, 1981) which indirectly may indicate an effect on testicular blood flow. There could be differences between LH and hCG regarding the circulatory effect on testis, since a large dose of hCG given as a single injection has a more prolonged effect on testicular steroidogenesis and metabolism than does LH (Lin et al., 1980; Moger, 1977).

In the present study we measured the effect of hCG on testicular blood flow using the radioactive microsphere technique (Damber and Janson, 1977). Since estrogens are known to inhibit the stimulatory effect of hCG on testosterone secretion (Saez et al., 1978), we studied effects of estradiol-17β on testicular blood flow. The purpose of the study was to clarify further the relationship between testicular blood flow and endocrine function.

MATERIALS AND METHODS

Animals and Anesthesia

Male rats (350–400 g) of the Sprague-Dawley strain (Anticimex, Sweden) were used. They were kept in a controlled environment and fed standard rat pellets and water ad libitum. Anesthesia was introduced by sodium pentobarbitone (Nembutal, Abbott, U.K.) in a dose of 40 mg/kg i.p. as a single injection. During each experiment the animals were kept supine on a heating pad.

Treatments

The rats were divided into four groups with 7–10

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animals in each group. The first group was treated with 0.1 ml peanut oil s.c. for 5 days (oil). In a separate experiment not reported here, this group did not differ in any respect from an untreated group of rats. The second group was given 50 μg estradiol-17β (Sigma, Sweden) in 0.1 ml peanut oil s.c. for 5 days (E_2). Measurements of blood flow and sampling of blood were performed the day after the fifth injection for these two groups of rats. The third group of rats received 100 IU hCG (Gonadex, LEO, Sweden) 20–24 h prior to the experiments (hCG). The fourth group of rats received the combination of the treatment given to groups two and three, that is, both estradiol-17β and hCG (E_2 + hCG).

Measurement of Testicular Blood Flow by Radioactive Microspberes

Blood flow to various organs was measured using radioactive microspheres (Damber and Janson, 1977) labeled with ^{141}cerium (15 μm diameter) and purchased from NEN, USA. A detailed evaluation of the microsphere method for testicular blood flow measurement in the rat has been presented (Damber and Janson, 1977). During the measurement and sampling period, systemic arterial blood pressure was measured by a catheter in the right brachial artery connected to a Sanborn Pressure Transducer 267 A connected to a Sanborn Transducer Amplifier-Indicator model 311 A. The blood pressure was recorded on a Devices model M2 Pen Recorder.

Measurement of Plasma Testosterone Concentration

An arterial blood sample was taken from the right carotid artery immediately following the blood flow measurement. Plasma from each sample was stored at −20°C until analysis. Plasma testosterone concentration was measured by radioimmunoassay (Damber and Janson, 1978a). The between-assay coefficient of variation was 14.7%.

Statistics

Comparisons between groups were made using Kruskal-Wallis one-way analysis of variance and Mann-Whitney U test (Siegel, 1956). A P value less than 0.05 was considered significant.

RESULTS

The infusion of microspheres did not affect the mean arterial blood pressure which was stable during the experiment. The number of microspheres found per unit of tissue weight in the right and left kidney was highly correlated (r = 0.76; n = 39), indicating an even distribution of microspheres between the right and left side of the body.

The effects of the different treatments on mean arterial blood pressure and blood flow to some organs are shown in Table 1. Analysis of variance gave no significant differences between groups for any organ, indicating that the effect seen on testicular blood flow is specific. Treatment with E_2 resulted in a significant decrease ($P<0.05$) in testicular and epididymal wet weights (Table 2). After hCG injection, a significant increase of testicular ($P<0.01$) and epididymal ($P<0.05$) wet weights was observed. A small increase of testicular wet weight ($P<0.05$) was also observed in the group treated with both E_2 and hCG (Table 2).

There was a significant increase ($P<0.01$) of testicular blood flow 20–24 h after hCG injection (Fig. 1). Treatment with E_2 alone did not change blood flow in the testis, but this treatment partly diminished the stimulatory effect of hCG ($P<0.05$).

After hCG injection, there was a significant ($P<0.01$) increase of testosterone concentration in peripheral blood (Fig. 2). E_2 treatment significantly ($P<0.01$) suppressed the testosterone concentration in plasma. An increase was seen in the group treated with both E_2 plus hCG ($P<0.01$) although the increase was smaller than for the group treated with only hCG ($P<0.01$).

With data from all four groups of rats, a correlation coefficient of r = 0.75 was found between plasma testosterone concentration (ng/100 ml) and total testicular blood flow (ml/min).

DISCUSSION

The results of the present study clearly demonstrate a vascular effect of hCG on the testis in addition to the marked steriodogenic effect of this hormone. The mechanism of action for hCG-induced increase in testicular blood flow is not known. A direct action on the testicular vessels seems unlikely since this would require specialized vascular receptors to distinguish this organ from other organs which were unresponsive to hCG. The vascular effect of hCG on the testis may be due to increased testicular metabolism and accumulation of vasodilative metabolites such as lactic acid, cyclic AMP, or steroids. Further support for this idea is the finding of a parallel reduction of blood flow and testosterone secretion in the rats treated with E_2 prior to the injection of hCG when compared with the rats treated only with hCG. On the other hand, even though E_2 treatment markedly depressed testosterone secretion, it did not affect the basal testicular blood flow. Furthermore, the effect of LH on testicular blood flow, in contrast to that of hCG, is small (Damber and Janson, 1978a) or

TABLE 1. Effect of estradiol-17β (E_2), hCG, and the combination of E_2 and hCG on mean arterial blood pressure and blood flow to some organs.

Treatment[a]	n	Mean arterial[b] blood pressure (mm Hg)	Blood flow to organs[b] (ml/100 g X min)			
			Epididymis	Kidneys	Small intestine	Liver
Oil	7	100 ± 5	17.6 ± 1.6	515 ± 30	157 ± 23	19.4 ± 4.1
E_2	8	98 ± 4	16.7 ± 1.2	449 ± 42	236 ± 43	25.8 ± 5.9
hCG	8	102 ± 9	16.6 ± 3.4	552 ± 80	166 ± 34	21.3 ± 7.7
hCG + E_2	9	96 ± 4	17.4 ± 2.6	431 ± 73	234 ± 52	23.6 ± 4.6

[a]For explanation see text.

[b]Values expressed as means ± SEM. Analysis of variance gave no significant difference between groups for blood pressure and blood flow to organs.

absent (Free, 1977; Nguyen-Duc-Kien, 1975; Amann et al., 1978) even though there is a pronounced effect on testicular steroidogenesis.

There appears to be a difference between the effect of LH and hCG on testicular blood flow. A single injection of hCG stimulates steroidogenesis for a longer period than an injection of LH (Lin et al., 1980; Moger, 1977). This may result in greater production and accumulation of vasodilative metabolites, thereby increasing oxygen demand and blood flow. The amount of oxygen present in vitro is of critical importance for the steroidogenic response to LH (Pirke et al., 1980).

The relation of the observed circulatory effect on the testis by hCG in the present study as well as the earlier observed effect of LH (Damber and Janson, 1978a) to physiological events is not certain. However, a relation between gonadotropic stimulation and testicular blood flow may exist since testicular blood flow per unit weight can be decreased after hypophysectomy (Setchell et al., 1969). Also, Pirke et al. (1979) found that the failure in the hypothalamic pituitary system observed in old rats leads to a decreased LH secretion, plasma testosterone concentration, and testicular blood flow, and that testicular blood flow was restored to normal upon hCG administration. A positive correlation was found between testicular blood flow and plasma testosterone concentration in the present study. In a previous report (Damber and Janson, 1978b), it was

TABLE 2. Wet weight of testes and epididymides after treatment with estradiol-17β (E_2), hCG, and the combination of E_2 and hCG.

Treatment[a]	n	Wet weight (mg)[b]	
		Testes	Epididymides
Oil	7	3551 ± 101	1209 ± 48
E_2	8	3326 ± 31*	1022 ± 15*
hCG	8	3819 ± 57**	1546 ± 20*
E_2 + hCG	9	3673 ± 50*	1161 ± 26

[a]For explanation see text.

[b]Values expressed as means ± SEM.

*P<0.05.

**P<0.01. Significantly different compared with control group.

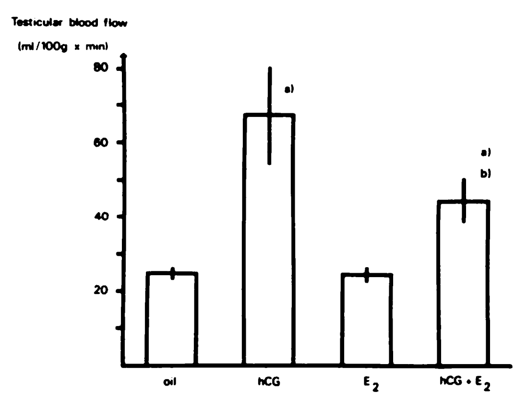

FIG. 1. Effect of estradiol-17β (50 μg/day for 5 days), hCG (100 IU), and the combination of E_2 and hCG on testicular blood flow. Values expressed as mean ± SEM. a) Significantly larger (P<0.01) than oil-treated control group. b) Significantly smaller (P<0.05) than the group treated with hCG.

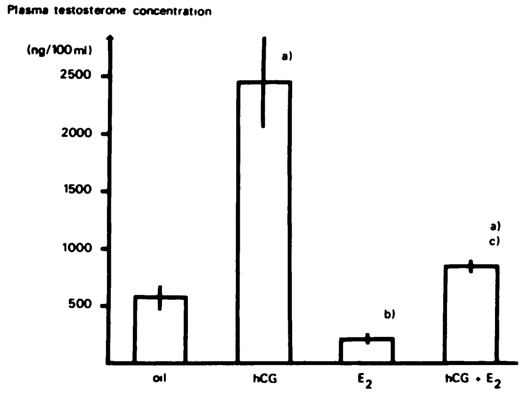

FIG. 2. Effect of estradiol-17β (50 μg/day for 5 days), hCG (100 IU), and the combination of E_2 and hCG on plasma testosterone concentration. Values expressed as mean ± SEM. a) Significantly larger ($P<0.01$) than oil-treated control group. b) Significantly smaller ($P<0.01$) than control group. c) Significantly smaller ($P<0.01$) than the group treated with hCG.

found that a strong positive correlation exists between testicular blood flow and output of testosterone in the spermatic vein. A tendency towards the same kind of relationship was observed by Free and Tillson (1973); Eik-Nes (1964) using an in vitro perfusion system demonstrated that the secretion of testosterone varied directly with the rate of perfusion.

That treatment with E_2 reduced the testosterone response to hCG agrees well with the report by Moger (1976) and Saez et al. (1978). The mechanism behind this observation could be a direct effect of E_2 on the Leydig cells or an inhibition of the LH-FSH secretion which may cause regression or degeneration of Leydig cells (Van Beurden et al., 1977). Wax et al. (1967) found a reduction of blood flow in the testis of dogs after treatment with polyestradiol phosphate. This is in disagreement with the present study where no effect on basal testicular blood flow was seen after E_2 treatment. One explanation for such discrepancy might be that Wax et al. (1967) used repeated treatment during a longer observation time. However, that E_2 treatment partly diminished the effect of hCG on testicular blood flow indicates a vascular effect of E_2 in addition to its marked inhibitory effect on testosterone production.

In conclusion, from the present study it appears that blood flow changes are involved in the effect of hCG on testicular endocrine function and that E_2 can partly inhibit these effects. Further studies are needed to clarify the temporal relationship between circulatory and metabolic events in the testis.

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