THE EFFECTS OF HCG, INDOMETHACIN, FLUFENAMIC ACID AND ASPIRIN IN THE IMMATURE FEMALE RAT

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Summary. Immature female rats (21 to 23 days old, 35 to 45 g) were injected subcutaneously with 2·5 i.u. HCG 18 hr before autopsy. Ovaries and uteri were removed; wet weight, dry weight and uterine protein content were determined. Ovarian and uterine weights, ovarian blood volume and uterine protein content were increased after HCG treatment. When immature female rats were pretreated with indomethacin, flufenamic acid or aspirin, the ovarian effects of HCG were inhibited: only slight increases in ovarian weight and blood volume were observed. Indomethacin attenuated the increases in uterine weight, and protein content, but neither flufenamic acid nor aspirin were effective in inhibiting these responses. The possible role of prostaglandins and of oestrogen as mediators of these responses is discussed.

INTRODUCTION

Administration of HCG initiates and supports a number of responses in the prepubertal female rat. These responses include: (a) the induction of ovulation (Zondek & Aschheim, 1927), (b) an increase in ovarian weight (Zondek, 1926), (c) an increase in uterine weight (Siddall, 1928), and (d) the production of an ovarian hyperaemia (Zondek *et al.*, 1945).

Ovarian hyperaemia is the earliest response, occurring in 2 to 18 hr and the enhanced blood flow presumably facilitates and underlies the ensuing events. Histamine has been implicated as a factor mediating the hyperaemia (Szego & Gitin, 1964). The large doses of antihistamine necessary to block the hyperaemia and the observation that hyperaemia can still be elicited by HCG in ovaries chronically depleted of histamine (Lipner, 1971) suggest, however, that histamine may not be an obligatory factor.

Since prostaglandins also modify blood flow in most vascular beds, including the ovary (Pharriss, 1971), as well as modify ovarian metabolism (Lamprecht *et al.*, 1973), steroidogenesis (Behrman *et al.*, 1971), and ovulation (Tsafiriri *et al.*, 1972), we have examined the effects of several inhibitors of prostaglandin synthesis on the ovarian hyperaemia after HCG treatment, and also the uterine

response to HCG administration during the early phase of HCG action and its modification by the inhibitors.

MATERIALS AND METHODS

Female rats, 21 days of age, of the Hebrew University strain were used; they were given free access to food and water. In all experiments, the animals were treated orally with 2.5 mg indomethacin (1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetic acid)/kg, 5 mg flufenamic acid (N(α,α,α trifluoro-m-tolyl)-anthranilic acid)/kg, or 25 mg aspirin (acetylsalicylic acid)/kg at 16.00 hours on Day 21, 10.00 and 16.00 hours on Day 22 and at 08.00 hours on Day 23. Control rats were fed the suspending vehicle (10% ethanol in propylene glycol) at the same times. Some of the rats in all treatment groups and the control group were given a subcutaneous injection of 2.5 i.u. HCG (Pregnyl; Organon) in saline at 16.00 hours on Day 22.

Ovarian blood volume (Exps 1 and 2) was determined by the distribution of ^{51}Cr -labelled red cells, which were treated according to the procedure of Sterling (1960) with 10 μCi ^{51}Cr (Radiochemical Centre, Amersham)/ml whole rat blood. Each animal received an injection into the tail vein of 0.5 ml blood with labelled red cells at 12.00 hours on Day 23 and was killed 1 hr later by an overdose of ether. The ovaries were removed, trimmed and an aliquot of venous blood was taken. Radioactivity was counted in a Packard 'Auto-gamma' Scintillation Spectrometer set for peak ^{51}Cr efficiency. The blood volume was calculated as the ratio of ct/min per pair of ovaries:ct/min per μl blood.

In Exps 3, 4, 5, 6 and 7, rats were given an overdose of ether at 11.00 hours on Day 23. The ovaries and uteri were removed, trimmed and weighed to the nearest 0.1 or 0.01 mg on a torsion balance (wet weights) or in tared vessels on an electronic balance (dry weights). Dry weights were measured the following morning after heating the samples in an oven at 80°C overnight. Protein was determined according to the procedure of Lowry *et al.* (1951).

Statistical calculations (analysis of variance) were performed with Dunnett's (1955) procedure for comparing several means to a single control or Tukey's (1949) method for testing all possible comparisons.

RESULTS

Ovary

Treatment with HCG elicited a marked rise in ovarian blood volume (Table 1). Treatment of the rats with indomethacin, flufenamic acid or aspirin resulted in an inhibition of this rise. There was a small decrease in ovarian blood volume in the rats treated with these drugs that was particularly noticeable after the treatment with indomethacin (Exp. 1), but the reduction was not statistically significant at the 5% level. At autopsy, the ovaries of the indomethacin-treated animals appeared pale and white, whereas blood vessels were always visible on the surface of ovaries from control rats and were par-

Table 1. The effects of HCG and three prostaglandin inhibitors on the ovarian blood content of immature rats

<i>Treatment</i>	<i>No. of rats</i>	<i>Ovarian blood content (μl/pair ovaries)</i>
Experiment 1		
None (control)	11	0.368 $\pm$ 0.047
HCG	14	0.557 $\pm$ 0.051*
HCG + indomethacin	8	0.302 $\pm$ 0.047
Indomethacin	9	0.245 $\pm$ 0.026
Experiment 2		
None (control)	5	0.392 $\pm$ 0.040
HCG	6	0.693 $\pm$ 0.060**
Flufenamic acid	5	0.300 $\pm$ 0.030
Flufenamic acid + HCG	5	0.410 $\pm$ 0.030
Aspirin	6	0.328 $\pm$ 0.030
Aspirin + HCG	6	0.350 $\pm$ 0.010

Values are Means $\pm$ 1 S.E.M.* Significantly different from control, $P < 0.05$.** Significantly different from control, $P < 0.01$.

ticularly prominent on the surface of ovaries from the animals treated with HCG.

In rats given HCG, the ovaries were visibly and measurably heavier by 18 hr after injection (Table 2). The effect of HCG was attenuated by the three inhibitors, which alone did not cause any substantial change in ovarian weight (Table 2).

Uterus

The effects of HCG and the various inhibitors on uterine weight are shown in Tables 3 and 4.

Table 2. The effect of HCG and three prostaglandin inhibitors on the ovarian weight of immature rats

<i>Treatment</i>	<i>No. of rats</i>	<i>Ovarian wet wt (mg/pair)</i>	<i>No. of rats</i>	<i>Ovarian dry wt (mg/pair)</i>
Experiment 3				
None (control)	15	15.50	11	3.03
HCG	12	20.50	10	4.40
HCG + indomethacin	11	18.80	11	3.50
Indomethacin	11	16.60	11	3.30
Experiment 4				
None (control)			6	2.54 $\pm$ 0.21
HCG			12	4.48 $\pm$ 0.25*
Flufenamic acid			12	2.77 $\pm$ 0.16
HCG + flufenamic acid			7	3.42 $\pm$ 0.29
Aspirin			6	2.33 $\pm$ 0.18
HCG + aspirin			7	3.26 $\pm$ 0.15

For Exp. 3, the values are averages derived from pooled weights. For Exp. 4, values are Means $\pm$ 1 S.E.M.

* Value is significantly different by Tukey's procedure from those for the Control, HCG + flufenamic acid and HCG + aspirin groups, $P < 0.05$.

Table 3. Effect of HCG and indomethacin on the uterine weight and protein content of immature rats

<i>Treatment</i>	<i>Wet wt of uterus (mg)</i>	<i>Dry wt of uterus (mg)</i>	<i>Protein/uterus (mg)</i>
Experiment 5			
None (control)	24.96 ± 1.26 (26)	5.07 ± 0.37 (16)	2.40 ± 0.40 (11)
HCG	38.49 ± 1.25** (30)	7.19 ± 0.36 (11)	4.61 ± 0.28 (11)
HCG + indomethacin	29.01 ± 1.08 (29)	5.60 ± 0.30 (16)	2.88 ± 0.18 (11)
Indomethacin	22.69 ± 0.59 (30)	5.56 ± 0.31 (16)	2.38 ± 0.08 (11)

Values are Means ± 1 S.E.M. Figures in parentheses represent the number of uteri.

** $P < 0.01$ compared with control.

Table 4. The effect of HCG, flufenamic acid and aspirin on the uterine wet weight of immature rats

<i>Treatment</i>	<i>No. of uteri</i>	<i>Uterine wet wt (mg/uterus)</i>
Experiment 6		
None (control)	20	21.9 ± 0.7
HCG	23	26.5 ± 0.8**
HCG + flufenamic acid	22	27.3 ± 0.9**
Flufenamic acid	17	22.9 ± 0.8
Experiment 7		
None (control)	6	19.3 ± 0.8
HCG	5	34.6 ± 2.5**
HCG + aspirin	6	36.7 ± 2.2**
Aspirin	6	20.2 ± 1.3

Values are Means ± 1 S.E.M.

** $P < 0.01$ compared with control.

In Exp. 5, there was a 54% increase in uterine wet weight 18 hr after HCG administration. The increase in wet weight was associated with comparable increases in dry weight and protein content and all these increases were inhibited when indomethacin was also given. For each parameter there was a small rise of 10 to 20% above control values but the increases were neither statistically significant nor of the same magnitude as those seen in the HCG-treated rats. Indomethacin by itself produced no discernible effect on uterine weight or protein content.

In Exps 6 and 7, neither flufenamic acid nor aspirin was effective in antagonizing the increase in uterine weight elicited by HCG (Table 4).

DISCUSSION

The factors mediating the ovarian weight gain and hyperaemia are only partly known. In hypophysectomized immature female rats, HCG produces a limited increase in ovarian weight which is confined to the ovarian interstitial tissue (Uma Bai *et al.*, 1973). In intact immature female rats the increase in ovarian weight produced by HCG appears to be due to a synergism with endogenous

FSH, the secretion of which may be stimulated by HCG (Evans & Simpson, 1950). The observation that the increase in ovarian weight that occurs in response to HCG was inhibited by indomethacin, flufenamic acid and aspirin suggests that at least part of the overall effect of these agents may be due to an inhibition of gonadotrophin release. Carlson *et al.* (1974) have found that indomethacin effectively blocks the release of LH in response to oestradiol infusion in the sheep.

Histamine has been implicated as a mediator of ovarian hyperaemia since (a) there is a marked depletion of ovarian histamine 2 to 2½ hr after the administration of LH to 21- to 22-day-old female rats (Szego & Gitin, 1964), and (b) benadryl (4 mg/kg) blocks the hyperaemia produced by HCG (Lipner, 1971). In contrast, HCG will still elicit hyperaemia in ovaries chronically depleted of histamine by compound 48/80 (a mixture of polymers of p-methoxy-n-methylphenylethylamine) (Lipner, 1971). Since prostaglandins have been shown to modify circulatory dynamics in a number of vascular beds (Bergström *et al.*, 1968; Weeks, 1972) and to stimulate several ovarian metabolic processes *in vitro* (Lamprecht *et al.*, 1973; Ahrén & Perklev, 1973), it appears reasonable to suggest that they are involved in the production of the ovarian hyperaemia. It is well established that indomethacin, aspirin and flufenamic acid all inhibit the synthesis of prostaglandins (Vane, 1971; Zor *et al.*, 1973), although they may have other actions as well. Each of these inhibitors blocked the increase in ovarian blood content in the rats treated with HCG in the present study, and it is likely that the prostaglandins are involved in the production of the ovarian hyperaemia. Direct confirmation of their role, however, remains to be determined.

The factor(s) mediating the increase in uterine weight seen in these experiments are not immediately obvious. It is evident that a uterotrophic agent is elaborated in the prepubertal female rat since there is a steady increase in uterine weight and histological differentiation from 21 to 42 days of age (Lunn & Lorraine, 1973; Somjen *et al.*, 1973). It has been shown that there are significant levels of circulating oestrogens in the immature female, although their origin is unclear (Weisz & Gunsalus, 1973). Thus, the increase in uterine weight elicited by HCG may be secondary to an enhanced secretion of oestrogen.

It is exceedingly difficult, however, to show the presence or synthesis of oestrogen in the prepubertal rat ovary either before or within 24 hr of the administration of HCG (Zondek & Sulman, 1945; Cierciorowska & Russfield, 1968). For example, Zondek & Sulman (1945), by castrating or administering anti-HCG serum to immature rats at specific times following HCG treatment, have shown that evidence can be obtained for the secretion of oestrogen only after 27 to 28 hr of exposure to HCG. In addition, the ovarian conversion of precursors to oestrogen *in vitro* is very low in the prepubertal rat (Vilée *et al.*, 1969; Quattropani & Weisz, 1973). Thus, the mediation of oestrogen in the responses reported here, which occur by 18 hr after HCG administration, remains undecided.

The site and mode of action of indomethacin in inhibiting the uterine weight increase produced by HCG remains unclear. Its action is probably not related to an inhibition of prostaglandin synthesis since neither flufenamic acid nor

aspirin duplicated this effect. It may, however, be related to a more general adverse effect of this drug in the rat, such as we have noted on the ovaries, and would dictate that caution be used in interpreting results with this drug.

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REFERENCES

- AHRÉN, K. & PERKLEV, T. (1973) Effects of PGE₁ and 7-oxy-13-prostynoic acid on the isolated prepubertal rat ovary. *Adv. Biosci.* **9**, 717–721.
- BEHRMAN, H., YOSHINAGA, K. & GREEP, R.O. (1971) Extraluteal effects of prostaglandins. *Ann. N.Y. Acad. Sci.* **180**, 426–435.
- BERGSTROM, S., CARLSON, L.A. & WEEKS, J.R. (1968) The prostaglandins: a family of biologically active lipids. *Pharmac. Rev.* **20**, 1–48.
- CARLSON, J.C., BARCIKOWSKI, B., CARGILL, V. & MCCracken, J.A. (1974) The blockade of LH release by indomethacin. *J. clin. Endocr. Metab.* **39**, 399–402.
- CIERCIOROWSKA, A. & RUSSFIELD, A.B. (1968) Determination of estrogenic activity of the immature rat ovary. *Archs Path.* **85**, 658–662.
- DUNNETT, C.W. (1955) A multiple comparison procedure for comparing several treatments with a control. *J. Am. statist. Ass.* **50**, 1096–1121.
- EVANS, H.M. & SIMPSON, M.E. (1950) Physiology of the gonadotrophins. In *The Hormones*, Vol. II, pp. 351–404. Eds. G. Pincus and K. V. Thermann. Academic Press, New York.
- LAMPRECHT, S.A., ZOR, U., TSAFRIRI, A. & LINDNER, H.R. (1973) Action of prostaglandin E₂ and of luteinizing hormone on ovarian adenylate cyclase, protein kinase and ornithine decarboxylase activity during postnatal development and maturity in the rat. *J. Endocr.* **57**, 217–233.
- LIPNER, H. (1971) Ovulation from histamine depleted ovaries. *Proc. Soc. exp. Biol. Med.* **136**, 111–114.
- LOWRY, O.H., ROSEBROUGH, N.S., FARR, A.L. & RANDALL, R.J. (1951) Protein measurements with the Folin phenol reagent. *J. biol. Chem.* **193**, 265–275.
- LUNN, S.F. & LORRAINE, J.A. (1973) The effect of initial age on the response of the rat to treatment gonadotrophins. *Acta endocr., Copenh.* **72**, 209–217.
- PHARRISS, B. (1971) Prostaglandin induction of luteolysis. *Ann. N.Y. Acad. Sci.* **180**, 436–444.
- QUATTROPANI, S.L. & WEISZ, J. (1973) Conversion of progesterone to estrone and estradiol in vitro by the ovary of the infantile rat in relation to the development of its interstitial tissue. *Endocrinology* **92**, 1269–1276.
- SIDDALL, A.C. (1928) A suggested test for pregnancy. *J. Am. med. Ass.* **90**, 380.
- SOMJEN, D., KAYE, A.M. & LINDNER, H.R. (1973) Postnatal development of uterine response to estradiol-17 β in the rat. *Dev. Biol.* **31**, 409–412.
- STERLING, K. (1960) Radioactive chromium technic for circulating red cell volume. *Meth. med. Res.* **8**, 69–75.
- SZEGO, C.M. & GITIN, E.S. (1964) Ovarian histamine depletion during acute hyperaemic response to luteinizing hormone. *Nature, Lond.* **201**, 682–684.
- TSAFRIRI, A., LINDNER, H.A., ZOR, U. & LAMPRECHT, S.A. (1972) Physiological role of prostaglandins in the induction of ovulation. *Prostaglandins* **2**, 1–10.
- TUKEY, J.W. (1949) Comparing individual means in the analysis of variance. *Biometrics* **5**, 99–114.
- UMA BAI, R., BISCHOFF, K., MACOME, J.C. & DICZFALUSY, E. (1973) *In vitro* sterol and steroid synthesis by ovaries of hypophysectomized immature rats treated with human gonadotrophins. *Acta endocr., Copenh.* **73**, 321–334.
- VANE, J. (1971) Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. *Nature, New Biol.* **231**, 232–235.

- VILLEE, C.A., CHANNING, C., ECKSTEIN, B. & SULOVIC, V. (1969) Effects of gonadotrophins on the metabolism of luteinized and follicular ovarian tissues. In *The Gonads*, pp. 277–294. Ed. K. W. McKerns. North-Holland Publishing Co., Amsterdam.
- WEEKS, J.R. (1972) Prostaglandins. *A. Rev. Pharmac.* **12**, 317–336.
- WEISZ, J. & GUNSALUS, P. (1973) Estrogen levels in immature female rats: true or spurious—ovarian or adrenal? *Endocrinology* **93**, 1057–1065.
- ZONDEK, B. (1926) Ueber die funktion des ovariums. *Dt. med. Wschr.* **8**, 343–344.
- ZONDEK, B. & ASCHHEIM, S. (1927) Hypophysenvorderlappenhormon und ovarium. Beziehungen der endokrinen Drüsen zur Ovarial funktion. *Arch. Gynäk.* **130**, 1–45.
- ZONDEK, B. & SULMAN, F. (1945) The mechanism of action and metabolism of gonadotropic hormones in the organism. *Vitams Horm.* **3**, 297–336.
- ZONDEK, B., SULMAN, F. & BLACK, R. (1945) The hyperemia effect of gonadotropins on the ovary. *J. Am. med. Ass.* **128**, 939–944.
- ZOR, U., BAUMINGER, S., LAMPRECHT, S.A., KOCH, Y., CHOBSIENG, P. & LINDNER, H.R. (1973) Stimulation of cyclic AMP production in the rat ovary by luteinizing hormone: independence of prostaglandins mediation. *Prostaglandins* **4**, 499–507.