THE EFFECT OF THE URINARY GONADOTROPHIN INHIBITOR ON THE RAT UTERINE WEIGHT RESPONSE TO HUMAN CHORIONIC GONADOTROPHIN

By

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ABSTRACT

A urinary extract (GIM), which previously had been shown to inhibit small doses of human chorionic gonadotrophin (HCG) in the mouse uterus assay, was tested in the rat. In this species, GIM caused an increase in the basal uterine weight and potentiated the response to 0.1 IU HCG. Similar results, and in addition augmentation of the activity of 0.2 IU HCG, were obtained in rats injected with carbon tetrachloride or starved. GIM inhibited the activity of 0.8 IU and 1.6 IU HCG. This was thought to result from the difference in mean final body weight between the GIM and the control groups.

The results support the hypothesis that GIM causes a non-specific stress reaction. In rats the effect of this is to increase endogenous gonadotrophin secretion. This contrasts with the results previously reported for mice, which suggest that stress suppresses endogenous follicle-stimulating hormone release.

A substance with gonadotrophin-inhibitory properties has been extracted from the urine of young children (Landau et al. 1960) and normal adult subjects (Soffer et al. 1962). The material inhibited the activity of pituitary luteinising hormone and human chorionic gonadotrophin (HCG) in the mouse uterus assay, but did not influence the activity of follicle-stimulating hormone (Futterweit et al. 1963; Soffer & Fogel 1963). When urinary extracts from normal subjects and from patients with various gonadal disorders were tested for gonadotro-
phin (HCG) inhibition, the results suggested that the inhibitor played a major role in the physiological control of sexual function as well as being implicated in the pathogenesis of certain diseases (Soffer et al. 1965; Fogel & Soffer 1967).

In an earlier investigation (Hipkin 1968), the effect of the urinary inhibitor on the activity of various gonadotrophins in the mouse uterus assay was studied. On the basis of the type of gonadotrophin affected and the degree of inhibition observed, it was postulated that the mode of action of the inhibitor was to suppress endogenous gonadotrophin secretion rather than the exogenously administered hormone. In order to obtain support for this hypothesis, it was decided to study the effect of the urinary inhibitor on HCG activity in intact and hypophysectomised rats. It has been claimed that the urinary inhibitor prevents the stimulatory effect of HCG on the weanling rat uterus (Soffer et al. 1966). Although this was confirmed, the results as a whole were completely different from those obtained in mice. They are reported because they explain several anomalies in the literature and cast doubt on the clinical importance of the inhibitor.

MATERIALS AND METHODS

Animals

Immature, female rats of the VS strain (colony of the Statens Seruminstitut originating from the Wistar strain) were used. They were 20-22 day old and initially weighed 35-43 g. Except for the starvation groups described below, all animals were given food and water ad libitum.

Materials

HCG (Physex®, Leo) was dissolved in distilled water. The total doses given ranged from 0.1 or 0.2 IU to 1.6 IU.

Gonadotrophin-inhibitory material (GIM) was extracted from pooled urine, from patients of either sex, by the kaolin-acetone method of Loraine & Brown (1959). The extracts were suspended in distilled water and boiled for 1 hour under reflux to destroy gonadotrophic activity. A total dose of five hundred ml urine equivalents was used.

Experimental procedures

The rats were divided into groups containing at least sixteen animals. They were injected subcutaneously with HCG on three successive days (0.5 ml daily). One group was injected with distilled water to provide the basal uterine weight. Half the animals in each group were then injected subcutaneously (but at a different site) with GIM (0.2, 0.2 and 0.1 ml). The remainder were treated in the same way with distilled water.

Two further series of experiments, similar to that above, were carried out. In the first, instead of GIM, the animals were given a single intraperitoneal injection (0.15 ml) of a carbon tetrachloride (CCl₄)/peanut oil mixture (1:1) at the time of the first injection of HCG. The results from this series were compared with those from animals given 0.15 ml peanut oil by the same route. CCl₄ was also tested for its effect on 0.5 and 1.0 µg oestrone. Oestrone was administered in propylene glycol, 0.1 ml daily. In
The other series of experiments, HCG assay was performed in rats starved from the time of the first injection, the results being compared with those from animals given food *ad libitum*.

The assay was also conducted in animals hypophysectomised by the parapharyngeal route. They were treated with HCG as above, the first injection being given immediately after the operation. The range of doses of HCG had to be extended to 25.6 IU in the hypophysectomised animals. The effect of GIM on the activity of 6.4 IU HCG in the hypophysectomised rat was also tested. In order to overcome the toxic effects of GIM in such animals, they were given cortisone acetate (Cortone®, Merck, Sharp & Dohme) 200 µg subcutaneously daily. It is most unlikely that this dose would affect HCG or GIM activity (*Hipkin 1970, in press*).

On the fourth day, the rats were killed. Mean body and uterine weights of the groups were compared using Student's *t*-test. Some results were compared by the method of *Gaddum* (1953). The sella turcica of the hypophysectomised animals was examined under a dissecting microscope for remnants of pituitary tissue. If these were present, the uterine weight for that animal was ignored.

**RESULTS**

The effect of GIM on HCG activity in the rat uterus assay is shown in Fig. 1. GIM itself caused an increase in uterine weight (*P* < 0.05). GIM also significantly potentiated the response to 0.1 IU HCG (*P* < 0.05). Although the

![Dose-response curves for HCG in rats treated with urinary inhibitor (---) and water (-----). se of mean indicated by vertical lines.](image)
response to 0.2 IU HCG was augmented, this was not significant. Using the responses to 0.2 IU and 0.4 IU HCG, the relative potency of HCG in the GIM treated rats was 1.01 (0.73-1.39) that in water treated control animals. On the other hand, GIM significantly inhibited the activity of 0.8 IU and 1.6 IU HCG ($P < 0.001$ and $P = 0.025$ respectively). Put in another way, GIM lowered the response to HCG in the plateau part of the dose-response curve. The mean final body weight of animals given water injections was 41.9 ± 0.4 g compared with 38.7 ± 0.7 g for the GIM treated groups. The difference was highly significant ($P < 0.001$).

The effect of CCl$_4$ administration (Fig. 2) and starvation (Fig. 3) on HCG activity in rats was similar to that of GIM. There was a significantly higher basal uterine weight in the CCl$_4$ treated animals compared with that in oil treated controls ($P < 0.02$). The responses to 0.1 IU and 0.2 IU HCG were augmented in CCl$_4$ treated rats ($P < 0.001$) while that to 0.8 IU was inhibited by the treatment ($P = 0.01$). There was no difference in response between groups given 0.4 IU HCG and this was also true for groups given 1.6 IU HCG. The potency of oestrone in CCl$_4$ injected rats was 1.3 (0.75-4.04) that in oil treated animals. There was a highly significant difference ($P < 0.001$) in mean final body weight between the CCl$_4$ and oil treated rats (35.5 ± 0.5 g and 41.6 ± 0.3 g respectively).

The basal uterine weight and the response to 0.4 IU HCG in starved rats

![Fig. 2.](image)

Dose-response curves for HCG in rats treated with CCl$_4$ (---) and peanut oil (-----). SE of mean indicated by vertical lines.
were not significantly different from those in controls given access to food (Fig. 3). However, starvation did augment the responses to 0.1 IU and 0.2 IU HCG ($P < 0.01$ and $P = 0.001$ respectively), whereas the effects of 0.8 IU and 1.6 IU HCG were inhibited ($P < 0.001$). There was of course a highly significant lower mean final body weight in the starved animals compared with those given food ($31.6 \pm 0.4$ g and $43.0 \pm 0.4$ g respectively).

There was a marked reduction in the potency of HCG in hypophysectomised rats compared with that in intact animals (Fig. 4). In the latter the smallest effective dose given was 0.2 IU HCG, while in hypophysectomised animals a dose of 6.4 IU was necessary. Although a mean uterine weight in excess of 100 mg was produced by treating intact rats with 0.8 IU HCG, this value was not attained in hypophysectomised animals even when the dose was increased to 25.6 IU.

When 6.4 IU HCG was injected into GIM treated, hypophysectomised rats, a uterine weight of $32.0 \pm 3.6$ mg was produced. This was not significantly different from that obtained when GIM was withheld ($40.4 \pm 5.0$ mg).

**DISCUSSION**

Using the mouse uterus assay, the inhibition of HCG activity by heated urinary gonadotrophic extracts detects the urinary gonadotrophin inhibitor (Soffer &
Fig. 4.  
Dose-response curves for HCG in hypophysectomised (---) and intact (----) rats.  
SE of mean indicated by vertical lines.

Fogel 1963). When the method was applied to urine from normal subjects and from patients with various gonadal disorders, the results suggested that the inhibitor might play a role in the control of the menstrual cycle as well as in the pathogenesis of such conditions as hypogonadotrophic hypogonadism and Stein-Leventhal syndrome (Soffer et al. 1965; Fogel & Soffer 1967). Soffer and his colleagues believed the gonadotrophin inhibition to be specific since the material did not affect the activity of follicle-stimulating hormone (FSH) or oestrone (Futterweit et al. 1963). On the other hand, a number of workers have doubted the presence or significance of a urinary gonadotrophin inhibitor.

Rosenberg et al. (1965) only demonstrated gonadotrophin inhibition when the extracts were toxic to the experimental animals. Toxicity was also noted in the experiments of Hipkin (1968), but on the basis of the degree of inhibition observed, and on the type of gonadotrophin affected, it was concluded that the urinary inhibitor (GIM) suppressed the endogenous gonadotrophin secretion necessary for the activity of certain gonadotrophins. Furthermore, a similar type of gonadotrophin inhibition was found when the assay animals were starved or given injections of carbon tetrachloride (Hipkin 1969a). To obtain support for this hypothesis it was decided to study the effect of GIM in intact and hypophysectomised rats. Soffer et al. (1966) extended their work to the rat and showed that inhibitory activity could also be detected when using this species. Their report was a reply to that of Hahn & Albert (1965) who, using
three different extraction procedures, were unable to demonstrate the inhibitor. 
Hahn & Albert (1965) concluded that »further studies of the urinary gonadotrophin inhibitor, facilitated by the exchange of inhibitor preparations among investigators, would seem highly desirable«.

In the present investigation, the effect of GIM on HCG activity in the rat uterus assay depended on whether the response to HCG was on the slope or plateau part of the dose-response curve. GIM augmented the response to 0.1 IU HCG but inhibited the responses to 0.8 IU and 1.6 IU HCG. The augmentation of 0.2 IU HCG was not significant, the large standard error being caused by one animal with a uterine weight of 108 mg. These results are completely different from those obtained in mice (Hipkin 1968). In this species, GIM inhibited HCG at doses producing responses on the slope of the curve (0.25 IU to 1.0 IU).

Several interpretations of the augmentation of HCG activity found in the present investigation are possible. Hahn & Albert (1965) noted that heated extracts, prepared by both the Albert (1955) and Loraine & Brown (1959) methods, augmented the effect of small doses of HCG in the rat uterus assay. Hahn & Albert (1965) mixed the extracts and HCG before injection and thought the resulting augmentation was non-specific, and similar to that found when gonadotrophins are mixed with various other materials. The injections of HCG and GIM were given at different sites in the present investigation, and the failure of GIM to augment a relatively small dose of HCG in hypophysectomised animals, makes it unlikely that the effect is caused by other non-specific factors. In any case, the apparent stimulation of uterine growth by GIM remains unexplained. Another possibility is that the potentiation of HCG activity is mediated by increased endogenous secretion of ACTH following the stress of GIM injection. In the mouse, exogenous ACTH augments the response to HCG but does not affect the basal uterine weight (Hipkin 1970, in press). The most likely explanation for the phenomenon is that, in the rat, GIM increases endogenous gonadotrophin secretion. This theory would meet the objections to the hypothesis of Hahn & Albert (1965). Furthermore the ovarian ascorbic acid depletion following the injection of »inert« urinary protein (Koed & Hamburger 1968) or of GIM (Hipkin 1969b) could also be explained on the basis of release of endogenous luteinising hormone (LH).

The second problem concerns the inhibition of HCG responses lying on the plateau part of the dose-response curve. A similar result was obtained previously (Hipkin 1966) when HCG assay was conducted in carcinogen treated rats. It was thought that the maximal uterine weight produced by HCG would be proportional to the final body weight of the animals. Since the carcinogen also inhibited normal body weight gain, it was postulated that this was the cause of the gonadotrophin inhibition. To test the hypothesis, the experiments were repeated but the carcinogen was given to animals 72 hours older than the
controls. At the conclusion of the experiment there was no difference between the groups in either body weight or response to HCG. GIM inhibited body weight gain in the present investigation and it is therefore likely that this lowered the maximal uterine weight response to HCG. Hahn & Albert (1965) did not detect any inhibition of responses to larger doses of HCG but on the other hand, they used heavier animals than in the present investigation, and the effect of the extracts on weight gain was relatively slight or absent. Soffer et al. (1966) demonstrated the effect of the urinary inhibitor on the weanling rat uterus using a single dose of HCG (0.6 IU). Judging by the uterine weight obtained in their controls, and by the results obtained in the present investigation, the response to this dose would lie close to or on the plateau part of the curve. There also appeared to be a final body weight difference between some of the inhibitor treated animals and controls. It has been pointed out previously (Hipkin 1969a), that even if animals gain weight normally, GIM is still capable of inhibiting the growth of individual tissues if this is accelerated for any reason. Therefore the gonadotrophin inhibition described by Soffer et al. (1966) is more likely caused by an interference with growth than by a specific substance.

The final problem posed by the results is the reason for the species difference in the effect of GIM on the responses to small doses of HCG. As mentioned earlier, GIM inhibited these doses in the mouse uterus assay. When GIM was tested against various gonadotrophins, the type of gonadotrophin affected and the degree of inhibition produced suggested that GIM suppressed endogenous gonadotrophin (probably FSH) secretion (Hipkin 1968). Since starvation and injections of CCl₄ produced similar effects, it was concluded that the gonadotrophin inhibition produced by GIM was a non-specific reaction to stress (Hipkin 1969a). The failure of GIM to inhibit small doses of HCG in the rat uterus assay cannot be ascribed to inadequate dosage since, to allow for body weight differences, the amount of GIM given to each rat (500 ml urine equivalents) was two to three times greater than that previously used for mice (Hipkin 1969b). This dose was toxic judged by the failure of the rats to gain weight normally and increasing the dose of GIM to 750 ml urine equivalents caused the death of at least one animal in each group. Starvation and injections of CCl₄ in rats produced an almost identical effect to that of GIM. CCl₄ did not significantly affect the uterine weight response to oestrone so the augmenting effect of the hepatotoxic agent on HCG activity is not a result of an alteration in endogenous oestrogen catabolism. The results in the starvation and CCl₄ experiments suggest that the postulated increase in endogenous LH secretion produced by GIM is also a non-specific reaction to stress. The potentiation of HCG activity found by Hahn & Albert (1965) occurred in animals which gained weight normally. A similar result was obtained with 1.5 l equivalents of a heated Johnsen (1958) extract (personal observation). However, as stated ear-
lier, body weight changes are an insensitive index of the toxicity of injected extracts.

The results of the assay in hypophysectomised animals show that endogenous gonadotrophin secretion is necessary for the uterine weight response to HCG. The similarity between the effect of hypophysectomy on HCG assay in rats (Fig. 4) and the HCG inhibition produced by GIM in mice (Fig. 1, Hipkin 1968), supports the hypothesis that in the latter species, GIM inhibits endogenous FSH secretion. The results in rats indicate that even if this occurs, suppression is incomplete. This problem may be resolved further by testing the effects of GIM in specific assay procedures.

Although boiled extracts have been used in the present investigation, gonadotrophin inhibition is a property of unheated urinary extracts (Landau et al. 1960) and the heat stability of the inhibitor is made use of to separate its effect from that of gonadotrophins. Therefore, although the uterine weight assay is useful for routine work when small doses of extract are used, if toxic doses are necessary, an assay involving hypophysectomiced animals may be preferable.

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