

EFFECTS OF HUMAN CHORIONIC GONADOTROPIN (hCG) ON PITUITARY PROLACTIN (PRL) IN THE LIZARD, *UROMASTIX HARDWICKII*

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ABSTRACT

A consideration of the data indicates that 45 IU human chorionic gonadotropin (hCG) treated lizard pituitaries, extracted after 30 and 60 minutes gave a mean crop-sac diametric response of 2.15 cm and 2.13 cm respectively in pigeons. The role of hCG in accelerating the synthesis of pigeon's milk is extra ordinary, and it seems that hCG exhibits this effect via lizard pituitary lactotrophs.

INTRODUCTION

Prolactin (PRL) has more actions than all other pituitary hormones combined. PRL is produced at sites out side the pituitary gland and thus may act as a hormone, by the classic endocrine pathway, and as a growth factor, neurotransmitter or immune regulator, in an autocrine-paracrine fashion (Christine *et al.*, 1998).

The pigeon crop-sac is a well known target tissue for PRL action. Bioassays regarded to be specific for PRL are based upon pigeon crop-sac stimulation activities of the hormone (Riddle *et al.*, 1933; Lyons *et al.*, 1935; Rivera *et al.*, 1967; Pukac and Horseman, 1984).

Most investigators still rely upon the various forms of the pigeon crop-sac assay to quantify PRL. Of these the intra-cutaneous pigeon crop-sac assay (Lyons *et al.*, 1935) is considered more sensitive than the systemic variety. Several studies have demonstrated that it is affected by non-specific irritants (Lahr *et al.*, 1942; Bern *et al.*, 1968; Bahn *et al.*, 1956). A highly sensitive bioassay with an excellent precision index, to quantify the different molecular forms of pituitary PRL was developed by Prada *et al.*, 1990 who administered PRL into the pectoral muscles of male doves.

In addition to numerous stressors (Hall, 1944; Krulich *et al.*, 1974; Euker *et al.*, 1975; Seggi *et al.*, 1975; Mattheij *et al.*, 1977; Frantz *et al.*, 1972 and Friesen, 1973), hypertonicity (Buckman *et al.*, 1973 and Ahmad *et al.*, 2002a) and hypotonicity (Ahmad *et al.*, 2002b) have been reported to induce crop-sac reaction.

Bioassay remains a necessary technique for evaluation of functional activity, which often bears no relationship to immunoreactivity. The gonadotropins pose a special problem because many responses to one hormone are modified by the concurrent action of others, and special conditions must be chosen to minimize this influence. The pigeon crop mucosal cells are homogeneous and the organ is structurally much less complexed than most other hormone – responsive target organs, such as the mammary gland or prostate (Nicoll, 1990).

For many purposes, particularly the measurement of gonadotropins in blood and urine, bioassays are simpler than more accurate radioimmunoassays (Vande Wiele and Dyrenfurth, 1973) and allow the uncertainties that attend *in vitro* methods to be avoided (Nicoll, 1990).

MATERIALS AND METHODS

Twenty-four pigeons 8-10 weeks of age belonging to white race were used for crop-sac test. The birds were obtained from local breeders and housed in the laboratory for 5 days keeping one pigeon in one cage. They were fed with millet and water *ad libitum*.

Twenty-four *Uromastix hardwickii* were used in this study. They were obtained from local suppliers and kept in the departmental laboratory (Ahmad and Taqawi, 1978a, 1978b and 1979). The lizards used were both males and females. Another group of twelve pigeons and twelve *Uromastix* were kept as controls for comparison.

Drug information:

The gonadotropins are used in therapy primarily for the treatment of infertility and cryptorchism. The widest potential usefulness of the gonadotropins is in the induction of ovulation in women who are infertile because of pituitary insufficiency. Extensive clinical experience with menotropins and hCG (Thompson and Hansen, 1977) indicated the occurrence of ovulation in 75 per cent of patients appropriately selected and treated with these drugs. While ovulation was occasionally seen during administration of menotropins before hCG was given. It usually took place about 18 hours after administration of the latter hormone. Pregnancy resulted in approximately 25 per cent of the patients. Of these the abortion rate was 25 per cent and foetal abnormalities occurred in 2 per cent. Twenty per cent of pregnancies resulted in multiple births; as 15 per cent twins and 5 per cent with three or more conceptions. More interestingly in another series, the male-to-female sex ratio in single births was 0.88, but only 0.43 for births of twins. The growth and development of children born of mothers receiving gonadotropin treatment have been normal (Hack *et al.*, 1970).

The only complications reported for this therapy have been excessive ovarian enlargement as a result of the maturation of many follicles. This in turn, leads to the release of multiple ova and multiple births. Ovarian hyperstimulation may be seen several days after the administration of chorionic gonadotropin in a few per cent of patients. In this condition the enlarged ovaries give rise to pain in the lower abdomen, and if there is bleeding into the peritoneal cavity, the pain is always. Under the latter circumstance, hospitalization and observation for ovarian rupture are required. Methods have been devised to avoid these complications (Brown *et al.*, 1969).

While the use of human gonadotropin, either from the pituitary gland or from menopausal urine, to promote fertility in the male is a field that has not been explored extensively. However men with hypopituitarism have been rendered fertile by this means (Gemzell and Kjessler, 1964; Mancini *et al.*, 1971 and Manna *et al.*, 2001).

hCG injection, U.S.P., is a preparation derived from the urine of pregnant women; is sold under various trade names. It is usually given intramuscularly in doses of 1000 to 4000 IU two or three times weekly for several weeks for the treatment of cryptorchism or hypogonadism in men, and in doses of 8000 to 10,000 IU, on the day following treatment with menotropins to evoke ovulation. It is available in vials containing 1000, 5000, 10,000 or 20,000 IU as powder with an ampule of suitable diluent. Gonadotropin of pregnant mare, serum has been used clinically to a

limited extent for 4 decades; however, no clear-cut indications have emerged and there are few guidelines to dosage.

Drug administration:

In experiment-1 a dose of 45 IU aqueous hCG was administered intravenously to each lizard of batch 1. For this purpose a median slit of about an inch was made in the abdominal integument. The incised skin was detached and stretched from the muscles on both the sides to make the abdominal vein visible for intravenous injection and 12 lizards were decapitated after 30 minutes following hCG administration. Each of the pituitary extracted was tagged for the preparation of a separate suspension for bioassay.

Similarly, in experiment-2 the amount of hCG and the method of injection adopted was the same; with the exception, that 12 lizards belonging to batch-2 were killed exactly after 60 min. following hCG administration. No sooner their pituitaries were extracted, suspensions were prepared and tagged.

Pituitary suspensions:

Suspension of each pituitary was prepared separately by grinding with an agate and a mortar in pyrogen free distilled water so that a total volume of each pituitary suspension was 0.4 ml. It was transferred by a hypodermic syringe to a tagged serum bottle and refrigerated during the bioassay which was not more than 4 days.

Assay procedure:

The procedure adopted for bioassay was that of Grosvenor and Turner (1958). Twelve pigeons were used in each experiment. Feathers overlying the crop were plucked off 8 hours before starting intradermal injections.

In this experiment 0.1 ml pituitary suspension belonging to 30 min. of a corresponding tag was injected intradermally in the geometrical centre of each half of the crop-sac. In the same way 0.1 ml pituitary suspension of 60 min were injected in the centre of each half of the crop repeatedly for 4 days according to the operational scheme (Ahmad *et al.*, 2002a, b).

The pigeons of control group were injected with the pituitary suspension of untreated lizards in the similar way for 4 days.

Diametric measurement:

The birds were killed on day 5. The skin was separated, before bisecting the crop-sac. The lining of each half was rinsed with tap water and all the adipose tissue was removed. Each half of the crop was then stretched against the light of a table lamp, fitted with 100 watt bulb; while another person measured thrice the proliferated, circularly opaque area with the help of a caliper.

RESULTS

Data indicates that the minimum crop-sac diametric response of 30 minutes extracted pituitaries was 2.14 cm; a maximum of 2.17 cm with mean of 2.15 cm. However, the minimum diametric response was 2.10 cm; maximum of 2.15 cm with mean expansion of 2.13 cm in 60 minutes extracted pituitaries (Table-2). Whereas, the control group when injected with extracts of untreated pituitaries produced a minimum and maximum crop-sac diametric response of 2.08 cm and 2.15 cm respectively with mean 2.12 cm (Table-1).

Table-1

Crop-sac diametric response following intradermal injections of 45 IU hCG per day for 4 days

Pigeons No.	Pigeon's weight (g)	Diametric response* (cm)
1	340	2.12
2	348	2.12
3	332	2.08
4	342	2.09
5	334	2.2
6	338	2.13
7	351	2.15
8	348	2.1
9	327	2.13
10	337	2.11
11	346	2.12
12	350	2.09
Mean	341 $\pm$ 7.73	**2.12 $\pm$ 0.03

*Each figure is the mean of right and left crop-sac diametric measurements.

**The mean of 24 measurements of crop-sac diametric response with $\pm$ SD.

The crop-sac diametric response of control group (Table-1), when compared with the crop-sac diametric response to pituitary extracts after 30 minutes, a significant increase in test group was observed ($p < 0.05$). Whereas, the increase in crop-sac diametric response to pituitary extracts after 60 minutes, in comparison to control group was non-significant, indicating a fall in PRL titre with 30 minutes gap.

The comparison of crop-sac diametric responses of both test groups showed a significant difference between the mean crop-sac diameters ($p < 0.05$).

DISCUSSION

Studies show (Liu *et al.*, 1997) that PRL suppresses gonadotropin-induced ovulation and disturbs the coordinated gene expression of tissue plasminogen activator (tPA) in the rat ovary. Immune female rats injected with 10 IU pregnant mare's serum gonadotropin stimulated follicle growth. The female rats given 50, 100 and 200 μ g of PRL, 48 hours before receiving injection of 7 IU hCG later showed no significant effect on ovarian weight. While animals receiving PRL after hCG treatment showed suppression of hCG-induced ovulation (Liu *et al.*, 1997).

According to a recent investigation (Lincoln *et al.*, 2001) PRL plays a priming role in the gonadal tissues during non-mating season and thereby facilitates gonadal reactivation. Whereas,

extensive clinical research (Thompson and Hansen, 1977) indicated that hCG with its widest potential is useful in induction of ovulation in women; infertile of pituitary insufficiency.

In a recent study Manna *et al.*, (2002) determined a role for epidermal growth factor (EGF) in modulating steroidogenesis. The effects of EGF (mEGF) exert through high-affinity binding sites in the adrenal and gonadal cells.

According to a latest study (Ahmad *et al.*, 2003) intradermal injections of hCG produced 2.28 cm mean crop-sac epidermal diametric response. Whereas, the same dose administered and pituitaries extracted after 30 and 60 minutes produced a mean diametric response of 2.15 and 2.13 cm respectively (Table-2).

Present data strongly suggest that in certain pathological states hCG behaves like the dopaminergic antagonistic drugs; which interfere with the regulation of PRL secretion (Ahmad *et al.*, 2001). Needless to say, the oral contraceptives (Macleod, 1976) and hypothalamus with increasing TRH is known to alters PRL titre (Ahmad *et al.*, 2003).

Table-2
Crop-sac diametric response to the suspension of lacertilian pituitaries extracted after 30 and 60 minutes of hCG administration

Pigeons No.	Pigeon's weight (g)	Diametric response (cm)	
		30 min.	60 min
1	328	2.16	2.14
2	342	2.14	2.1
3	348	2.15	2.15
4	336	2.14	2.13
5	332	2.16	2.15
6	341	2.16	2.13
7	350	2.15	2.13
8	353	2.15	2.14
9	328	2.16	2.12
10	335	2.15	2.14
11	346	2.17	2.15
12	345	2.14	2.13
Mean	340 ± 8.48	*2.15 ± 0.01	**2.13 ± 0.01

*The mean of 12 measurements of the left crop-sac with ± SD.

**The mean of 12 measurements of the right crop-sac with ± SD.

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