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THE EFFECT OF VARYING CONCENTRATIONS OF ERGOMETRINE MALEATE ON THE PITUITARY PROLACTIN OF THE LIZARD, *UROMASTIX HARDWICKII*

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ABSTRACT

Present study deals with the effect of ergometrine maleate on prolactin (PRL) of *Uromastix* pituitaries. Administration of 0.02, 0.04 and 0.06mg/day/animal ergometrine maleate for 3 days to individuals of 3 respective groups resulted in decreased pigeon crop-sac diametric response. Intramuscularly treated pituitaries showed more PRL inhibition than the pituitaries of animals treated intravenously.

INTRODUCTION

Prolactin (PRL) has been reported to be present in the pituitaries of a number of higher and lower vertebrates (Azimov *et al.,* 1938; Dawson, 1946, 1948; Harlant, 1952a, Harlant *et al.,* 1957; Pearse, 1951 and Li, 1974). PRL shown to be present in the pituitaries of reptiles has been reported to be associated with the regulation of skin moulting, stimulation of somatic growth, stimulation of caudal regeneration and hyperphagia (Bern and Nicoll, 1969).

It has been demonstrated that administration of ouabain (Ahmad *et al.,* 2002) and complex peptides containing ergot alkaloids depress PRL (Berde *et al.,* 1978; Delitala *et al.,* 1980; Mann *et al.,* 1981; *Ravault et al.,* 1977; Gracia *et al.,* 1977; Ambrosi *et al.,* 1977 and Peter *et al.,* 1977) in man and mammals.

Therefore, the potent prolactin inhibiting activity of the ergots prompted their use in lactating rats and in rats bearing mammary tumors. Ergocornine (Nagasawa *et al.,* 1970; Clemens *et al.,* 1972 and Cassel *et al.,* 1971) and ergocryptine (Cassel *et al.,* 1971) were able to inhibit mammary tumor growth and development. 2 bromo-α-ergocryptine, a synthetic derivative of ergocryptine was able to inhibit lactation (Fluckiger *et al.,* 1968) and mammary tumor growth (Henson *et al.,* 1970 and Stahelin *et al.,* 1971).

Interestingly, the spiny tailed lizard, *Uromastix hardwickii* due to its low cost and economy of maintenance has been used in this study for the determination of PRL inhibiting capacity of ergometrine maleate.

MATERIALS AND METHODS

Assay animals:

Pigeons 8 to 12 weeks of age and weighing 250 ± 20 g and of mixed races were used as assay animals. Pigeons were housed one to a cage and were fed grain and water *ad libitum*. The birds were kept in laboratory not more than two days including the days of bioassay. The time of bioassay and the arrival of birds were synchronized.

Animals:

For the present investigation, experimental animals were obtained from local suppliers and were maintained at a temperature, 29 ± 1°C. They were divided into similar groups of control and test for experiment I and II (Table 1, 2).

Administration of drugs:

In experiment I, there were I-IV groups, comprised of 5 *Uromastix hardwickii* each, were administered ergometrine maleate (Novartis, Pakistan) intravenously. Each individual of group II was administrated 0.02mg of the drug per day for 3 days through the anterior abdominal vein. In the same way each individual of group III was administered 0.04 mg of the drug intravenously for 3 days. Individuals of group IV were administered 0.06 mg ergometrine maleate similarly for the same number of days. Animals of group I served as untreated controls.

Experiment II was formed of I-IV groups; each comprised of 5 individuals. Animals of the group I were kept as control. Animals of group II, III and IV were administered doses of 0.02, 0.04 and 0.06 mg ergometrine maleate in the hind limb of each individual for 3 days respectively.

Removal of Pituitaries:

On day 4, animals of control and test groups were decapitated one by one. The skull was cleaned and brain was scooped out. The pituitary was extracted from sella turcica by cutting the diaphragm sella (Ahmad *et al.,* 2002). Each pituitary kept in a marked vial was either preserved in the freezing compartment of a refrigerator for later assay or was used for the assay at once (Ahmad *et al.,* 2001a, b). However, the frozen and fresh pituitaries gave similar results.

Pituitary suspension:

A suspension was made by grinding the pituitaries with an agate and a mortar in a small but fixed quantity of pyrogen free distilled water. With the help of a hypodermic syringe, the suspension was transferred into serum bottle and stored in the refrigerator for injection during the bioassay (Ahmad *et al.*, 200la).

Assay procedure:

Assay procedure was essentially the same as that of Grosvenor and Turner (1958). Feathers of pigeons were plucked off from the skin overlying the crop-sac six to eight hours before starting the injections. Plucking the feathers, however, produces some relatively negligible proliferative response in the crop-gland (Hall, 1944, Ahmad *et al.*, 2002). A normal suspension of pituitary was injected intradermally on one side of the crop in volume of 0.1 cc per day for four days. An identical volume (0.1 cc) of pyrogen free water was injected on other side of the crop in the same bird serving as control. The suspension prepared of the pituitaries of treated *Uromastix* was similarly injected intradermally on one side of the crop. The injections were made with a 1 ml hypodermic syringe bearing a 27 gauge needle at the geometrical centre of each crop-gland (Hall, 1944). The site for insertion of needle had been marked previously with a non-toxic dye for subsequent reference. The injections were made in such a way that an intradermal bleb was always formed. The same procedure for each assay was adopted for experiment I, II.

The birds were killed on the day 5, twenty-four hours after the last injection. The skin was separated from the underlying crop-sac and the whole crop-sac was removed and bisected. The lining of each half was rinsed with running tap water and adherent fat was removed. Each half was stretched by one person against light of table lamp fitted with a 100 watt bulb as a source of light; while another person measured in centimeters with a caliper, the diameter of the proliferated area. When the stretched crop-sac is viewed against light, the proliferated epithelium appears as an

essentially circular opaque area of parallel epithelial strands.

RESULTS

Table 1 indicates that 2.35cm was the maximum crop-sac diametric response in controls. On the contrary, the suspensions of intravenously treated test groups II, III and IV showed comparatively low and varied responses. Injections of 0.06 mg ergometrine maleate produced least suppression of PRL in *Uromastix* pituitary; test group given 0.02 mg showed greater suppression than test group given 0.04 mg/day.

Table 1
Response of pigeon crop-gland to homogenates of 4 types of *Uromastix* pituitaries of normal and 3 different dose groups (II-IV) following intravenous administration of methyl ergometrine maleate

No. of groups	Animals/ Group	Dose injected (mg)	Total drug injected/ individual (mg)	Mean crop-sac response (cm) ± SD
I	5	Normal	Nil	2.35 ± 0.15
II	5	0.02	0.06	1.33 ± 0.40*
III	5	0.04	0.12	1.51 ± 0.36*
IV	5	0.06	0.18	1.66 ± 0.03**

*p < 0.01, **p < 0.005

In the same way, the homogenates of intramuscularly treated pituitaries gave different diametric responses. The least dose showed greatest PRL inhibition. The other dose showed almost similar pattern of PRL inhibition (Table 2).

Table 2
Response of pigeon crop-gland to homogenates of 4 types of *Uromastix* pituitaries of normal and 3 different dose groups (II-IV) following intramuscular administration of methyl ergometrine maleate

No. of groups	Animals/Group	Dose injected (mg)	Total drug injected/ individual (mg)	Mean crop-sac response (cm) ± SD
I	5	Normal	Nil	2.35 ± 0.15
II	5	0.02	0.06	1.33 ± 0.04**
III	5	0.04	0.12	1.37 ± 0.12*
IV	5	0.06	0.18	1.57 ± 0.10**

*p < 0.01, **p <0.005

When the mean crop-sac diameter of control group was compared with the mean diameters of test groups at varying doses by t-test, a significant difference ($p<0.01$ and 0.005) was observed, irrespective of the route of drug administration. Although the drug showed an inhibition of pituitary PRL, but intramuscular route is more effective in reducing the mean crop-sac diameter than intravascular route (Fig. 1).

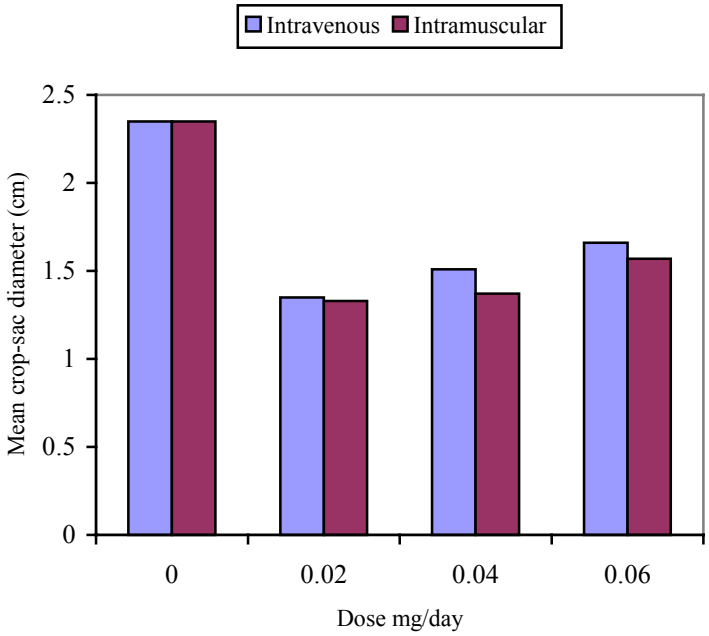

Fig. 1: Dose dependent crop-sac diametric response of pigeons to the pituitary homogenates of *Uromastix.*

The comparison of multisample means by two-way ANOVA showed a significant difference between the effect of doses of 0.02, 0.04 and 0.06 mg/day ($p<0.05$); moreover the effect of drug administered by two different routes differ significantly in suppressing the pituitary PRL ($p<0.05$).

DISCUSSION

Present investigation demonstrates that intravenous and intramuscular treatment with varying doses of ergometrine maleate caused a decrease in the pituitary PRL of *Uromastix hardwickii.*

A comparison of the crop-sac response of the lesser drug dose either given intravenously or intramuscularly showed almost no difference (Fig. 1). Intravenous injection of 0.02 mg ergometrine maleate treated lizard pituitaries inhibited PRL significantly, whereas homogenates of intramuscularly treated pituitaries showed comparatively greater inhibition. Moreover, pituitary suspension of both groups receiving 0.04 mg ergometrine maleate showed intermediate inhibition between low and high doses; moreover the administration of a high dose (0.06 mg/day) either intravenously or intramuscularly suppressed the pituitary PRL to a lesser extent in comparison to control. Evidence has been presented by Platt (1976) that PRL secretion is suppressed by ergocornine in neotenic *A. tigrinum*.

In a recent study (Ahmad *et al.,* 2003) ergometrine maleate accelerated the growth of *Rana cyanophlyctis* tadpoles. This indicates that ergometrine enhances the secretion of growth hormone.

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