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Research Article

# Effect of para-chlorophenylalanine on hormonal profile in anestrus buffaloes*

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## ABSTRACT

Parachlorophenylalanine (PCPA), when administered intra-peritoneally (100mg/kg body wt., I/P) is effective in reducing the level of prolactin(PRL) to certain extent by inhibiting serotonin biosynthesis in anestrus buffaloes. 5-hydroxytryptophan (5-HTP) (1mg/kg/body wt., I/M.) induces prolactin release by restoring serotonin biosynthesis. Level of prolactin increased about three folds as compared to pre-treatment levels and more than 10 folds as compared to PRL level at 32 hr after PCPA treatment. These compounds have effect on cortisol level of which showed fluctuations while as LH levels remain more or less unaffected. These compounds do not improve anestrus condition in buffaloes.

**Key words:** Anestrus Buffalo, Parachlorophenylalanine, 5-hydroxytryptophan, prolactin, Cortisol, LH.

## INTRODUCTION

Hypothalamo-hypophyseal-gonadal axis plays a major role in the regulation of reproductive cycle in buffaloes. While as, Adrenals are implicated to have some effect on reproductivity as cortisol and also that of prolactin level have been observed to change in relation to level of stress and stages of estrous cycle. Hyperprolactinimea has been reported to be associated with the anestrus condition in buffaloes during summer (Heranjal *et. al.* 1979b; Kaker *et. al.* 1982). Attempts to block total prolactin release in order to restore cyclicity in buffaloes is well documented, but blocking of stress induced prolactin release has never been attempted. In order to decrease the stress induced prolactin levels and its effect on hypothalamo-hypophyseal-gonadal axis and adrenal activity, parachlorophenylalanine has been used. It lowers the prolactin release by blocking serotonin (5HT). The seratogenic pathway of PRL release is again restored by administering 5HTP.

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## MATERIAL AND METHODS

Three summer anestrus buffaloes were administered parachlorophenylalanine intraperitoneally, @ 100 mg/kg body wt., as suggested by Sartin *et. al.* (1987) for steers. After 92 hr of this injection 5-hydroxytryptophan (5-HTP) @ 1mg/kg body wt., as suggested by Sugawara *et. al.* (1989) for goats, was administered intramuscularly. During the experimental period feeding and watering regimen as practiced at NDRI was followed.

### Collection and Processing of Blood

Blood samples from buffaloes treated with PCPA were collected in heparinized tubes at -24, -20, -16, -12, -8, -4, 0, +4, +8, +12, +16, +20, +24, +28, +32, +36, +40, +44, +68 and +92 hrs with respect to treatment provided. After 5-HTP administration, samples were collected at 2, 4, 6, 8, 10, 12, 24 and 48 hr. Blood samples were immediately carried to the laboratory in ice and were centrifuged at 3000rpm for 20min to separate

plasma. Plasma was stored frozen at -20°C till analysis.

**Hormones, Antibodies and Chemicals for Assay system**

Highly purified bLH (USDA-bLH-I-1) was obtained from USDA Reproduction Lab., Beltsville, MD, AFP6000, USA, and bovine prolactin (AFP-4835B) from Dr. A.F. Parlow, Scientific Director, NHPP, Harbor-UCLA Medical Centre, 100 West Carson St. Torrance, California 90509, USA. LH antiserum (bLH-MLM 343) was provided by Dr. Sujata Pandita, DCP Division, NDRI, Karnal and antiserum for prolactin (AFP-753180) by Dr. A.F. Parlow. Second antibody (GARGG), Cortisol (Hydrocortisone, 11 â, 17á, 21-Tryhydroxypregn-4-ene-3, 20-Dione), Antiserum for Cortisol, Dextran T-70, Parachlorophenylalanine, 5-HTP and bovine serum albumin were purchased from Sigma Chemical Company, St. Louis. USA. Isotope ($^{125}I$) for iodination of pure hormones was purchased from BARC, Mumbai. (1, 2, 6, 7- 3H) Cortisol was purchased from Amersham International PLC, Amersham. U. K. Other chemicals required in RIAs were procured from reputed local firms.

**RIA of Hormones**

Radio iodination of bLH and Prolactin was carried out by the method of Niswender et.al. (1969) using chloramine T. These hormones were estimated by RIA. Cortisol in blood plasma was determined by RIA technique described by Henericks *et. al.* (1984) and Sujata (1987).

**Recovery of Purified hormones**

Recovery of LH in the assay system varied from 95 to 108.67%, for prolactin the percent recovery was 96.98 to 108.48 where as for cortisol it was 95 to 98.43.

**Intra- and Inter- assay coefficient of variations**

Intra and inter assay variations for LH, PRL and cortisol were 3.78-8.89 and 8.15- 9.95; 3.19-10.32 and 7.16-8.19; and 5.43-10.54 and 10.20-11.25, respectively.

**Statistical analysis**

The data was subjected to appropriate statistical analysis to draw scientific inference. Means and SE were calculated, least square analysis performed and correlations were worked out as described by Snedecor and Cochran (1967).

## RESULTS AND DISCUSSION

Mean circulatory levels of LH, Prolactin and cortisol, pre-treatment, after PCPA treatment and 5-HTP treatment have been presented in table 1.

In castrated, estradiol-implanted female rats, circadian fluctuations in LH plasma levels are almost completely abolished 24hr after treatment with PCPA, and totally eradicated after 48 hr. Under these conditions, restoration of endogenous levels of the transmitter by administration of the immediate precursor of the amine 5-HTP at 0900hr restores the capacity of the hypothalamo-hypophyseal unit to release LH in the afternoon. This response can be obtained by systemic as well as by intraventricular administration of small doses of 5-HTP and is not strictly related to the time of injection (Hery *et. al.* 1976). Further more, in cyclic rats the PCPA administration leads to ovulatory failure (Hery *et. al.*, 1975). But in our study levels of LH did not differ significantly during pre-treatment and after PCPA and 5-HTP administration, when the data was compared irrespective of treatment to find out between treatment effects, if any. When same data were compared treatment-wise; to see between sampling intervals effect, then also there was no significant change. Parachlorophenylalanine (PCPA) was discovered by the observation of its effect on the endogenous levels of serotonin and is the most effective inhibitor of tryptophan hydroxylase. It was demonstrated that the observed decrease in serotonin level was brought about by irreversible inhibition of tryptophan hydroxylase. In their experiments on rats, Jorgensen *et. al.* (1992) have concluded that the serotonergic neurons are involved in the meditation of the stress induced prolactin release by activation of 5HT-1, 5HT-2 as well as 5HT-3

**Table 1: Mean circulatory levels of hormones in anestrus buffaloes before and after treatment with PCPA and 5-HTP**

| Treatment | Hour of sampling | Hormones | | |
|---|---|---|---|---|
| | | LH (ng/ml) | Prolactin (ng/ml) | Cortisol (ng/ml) |
| Pre-treatment | -24 | 0.29±0.16 | 42.01±1.22 | 0.85±0.36 |
| | -20 | 0.16±0.03 | 39.40±9.97 | 0.10±0.01 |
| | -16 | 0.23±0.03 | 45.43±3.01 | 0.14±0.12 |
| | -12 | 0.28±0.17 | 41.20±10.01 | 0.24±0.16 |
| | -8 | 0.25±0.12 | 35.58±9.91 | 0.08±0.08 |
| | -4 | 0.21±0.06 | 41.30±8.18 | 0.60±0.46 |
| PCPA administration | 0 | 0.14±0.08 | 60.29±6.16 | 2.11±1.38 |
| | +4 | 0.29±0.19 | 69.74±34.53 | 2.29±1.31 |
| | +8 | 0.78±0.48 | 46.07±12.57 | 1.06±0.62 |
| | +12 | 0.25±0.08 | 42.13±4.86 | 0.77±0.62 |
| | +16 | 0.57±0.10 | 45.15±11.46 | 1.15±0.32 |
| | +20 | 0.14±0.08 | 32.25±6.17 | 1.91±0.18 |
| | +24 | 0.16±0.04 | 29.24±4.57 | 4.60±0.81 |
| | +28 | 0.21±0.07 | 18.67±3.03 | 1.73±0.83 |
| | +32 | 0.12±0.06 | 12.81±4.63 | 2.75±1.37 |
| | +36 | 0.18±0.04 | 19.19±10.66 | 3.29±1.10 |
| | +40 | 0.42±0.19 | 26.31±7.82 | 7.41±3.80 |
| | +44 | 0.19±0.01 | 19.93±8.17 | 3.30±1.40 |
| | +68 | 0.45±0.35 | 56.02±14.64 | 5.12±1.77 |
| | +92 | 0.15±0.07 | 23.47±2.17 | 3.24±1.50 |
| 5-HTP administration | 2 | 0.14±0.05 | 159.63±29.21 | 2.01±0.59 |
| | 4 | 0.23±0.07 | 104.47±6.72 | 2.17±1.08 |
| | 6 | 0.09±0.02 | 51.54±3.55 | 1.91±1.10 |
| | 8 | 0.14±0.05 | 33.79±3.34 | 2.96±2.47 |
| | 10 | 0.06±0.03 | 36.36±8.21 | 1.67±0.66 |
| | 12 | 0.17±0.08 | 29.63±4.88 | 0.66±0.55 |
| | 24 | 0.25±0.12 | 45.77±16.16 | 1.87±1.01 |
| | 48 | 0.14±0.06 | 40.51±20.74 | 0.85±0.51 |

receptors. Findings of present study suggest similar mechanism of prolactin release in buffaloes also. There was a significant ($P<0.01$) period effect on the levels of prolactin, the overall mean values decreased after PCPA administration which again increased after 5-HTP administration as compared to the pre-treatment values. There was no significant animal to animal variation in the prolactin levels. The decrease in prolactin was visible between 28 and 36 hr of PCPA administration in different animals but it was not significant. It appeared that the dose of one single injection of PCPA was insufficient to bring the levels of prolactin down to a significant level. The

treated buffaloes did not exhibit any sign of estrus which was further confirmed by low levels of LH during the observation period. In rats, Mulloy and Moberg (1975), Scapagnini *et. al.* (1978), Jahn and Deis (1987) and Pan and Gala (1987) demonstrated that intra-peritoneal administration of PCPA depressed prolactin release, which was due to the blockage of 5-HT, that is responsible for PRL release. This was further validated by significant ($P<0.01$) increase in prolactin levels in all buffaloes, after 5-HTP administration that was highest at 2 hr of sampling, followed by a decrease to reach minimum values at 8 to 10 hr. thereafter, the values fluctuated within narrow limit. Parachlorophenylalanine also eliminated the diurnal fluctuations of plasma corticosteroids in birds, rats and cats (Muller *et. al.*, 1977). In rats, treated with the soluble form of PCPA-methyl ester (PCPA m.e.), the abolition of the rhythm was difficult to observe (Rotszejn *et. al.* 1977). However, in comparable experiments, it was demonstrated that the soluble form of PCPA @300mg/kg body wt. given 48hr earlier or PCPA m.e. @200mg/kg body wt. given 24hr earlier resulted in constant plasma B (corticosteroid) levels throughout the day that were higher than normal in the morning, but lower than normal in the evening (Scapagnini *et. al.* 1978). In present study the findings are bit different. There was highly significant ($P<0.01$) animal to animal variation in the cortisol levels, after PCPA and 5-HTP administration. There was highly significant ($P<0.01$) period effect as cortisol levels fluctuated on different sampling hours.

The values were highest after PCPA administration, followed by 5-HTP administration and least during pre-treatment period. There was no definite trend for change in cortisol levels in relation to the administration of PCPA and 5-HTP.

It may be concluded from the above findings that single I/P injection of PCPA @ 100mg/kg body wt, though decreases the prolactin level in buffaloes, it is not sufficient to abolish it completely from the system. Furthermore, partial decrease in prolactin through PCPA injection does not improve cyclicity in anestrus buffaloes and serotonergic pathway is involved in prolactin release in buffaloes.

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