Cytological Study on the Anterior Pituitary of Beagle Bitches Treated Subcutaneously with Progesterone for 13 Weeks

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Abstract. The effects of subcutaneous administration of 25 mg/kg/day progesterone for 13 weeks on the pituitary glands of immature ovariectomised and mature intact beagle bitches were studied using a histochemical technique. In the treated animals the most striking effect was an apparent increase in the relative number of prolactin producing cells (PRL cells) when compared with the controls, whether mature in the anoestrous or prooestrous phases of the cycle or immature (intact or ovariectomised).

While the relative number of PRL-producing cells in the progesterone-treated bitches was comparable to that in the controls in the metoestrous phase of the cycle, in the former they showed more morphological signs of intense secretory activity, accompanied in most cases by a rise in serum prolactin levels. The accelerated secretory activity of PRL cells accounts for the capacity of progesterone to induce excessive mammary gland development.

In the progesterone treated bitches and in the control animals in the metoestrous phase of the cycle, the STH cells diminished in number. Moreover in these animals, the number of STH and ACTH cells showing morphological signs of secretory activity was slightly more than in the other animal troupes.

The gonadotrophs exhibited involutionary changes under treatment with progesterone.

Key words: Pituitary gland — Beagles — Mammary glands — Progesterone.

Introduction

Hypophyseal reaction to progesterone or synthetic progestogen treatment in mammals is not fully understood. Contradictory results have been obtained in women after prostegational treatment, however the experimental design and dosage regimen in these studies were not comparable.

While some investigators concluded that the prostegational treatment did not affect the prolactin levels in women (Jaffe et al., 1973; Beck and Malarkey, 1976;
Bukman and Peake, 1973), others showed the opposite (Dericks and Taubert, 1976; Chaudhury et al., 1977; Daniel et al., 1977).

Since the bitch has been used as a model in long term toxicity studies to assess the side-effects of contraceptive steroids, great interest has been focussed on this species.

There have been few studies published recently concerning the effect of synthetic progestational hormones on the canine pituitary gland (El Etreby et al., 1973; El Etreby and Fath Elbab, 1977), and to our knowledge, the action of natural progesterone is lacking in the available literature. In order to be able to interpret results obtained after treatment with synthetic progestogens, knowledge of the action of natural progesterone on the cellular elements of the anterior pituitary gland is deemed necessary. Therefore, the present study was designed to assess the physio-pathological changes of the cellular elements of the anterior pituitary (AP) under the influence of daily administration of progesterone for 13 weeks.

Materials and Methods

A group of three immature ovariectomised and another of two mature intact bitches in the anoestrous phase of the cycle, were given daily subcutaneous injection of progesterone at a dose level of 25 mg/kg/day for 13 weeks. The progesterone was dissolved in the oily vehicle (benzyl alcohol, 10 ml; benzyl benzoate, 90 ml; ethyl oleate, 100 ml) in a concentration of 100 mg/ml.

Three other groups consisting respectively of two immature intact, two immature ovariectomised (one was killed during the experimental period due to rectal prolapse) and three mature intact bitches in the anoestrous phase of the cycle, received the corresponding daily volume of the vehicle only, and acted as controls. By the end of the dosing period, one bitch from the latter group was in metoestrus, another in prooestrus and the third in anoestrus. Moreover, for comparative purposes, pituitary glands of bitches in the different phases of the oestrous cycle (two bitches per phase) were included. The dose was adjusted once a week in accordance with body weight changes. Clinical observations were conducted daily and the animals were controlled for signs of oestrus thrice weekly.

The mammary glands were examined for development weekly by inspection and palpation. Sera were collected and deep frozen at -70 °C at 3-weekly intervals during the dosing period. Serum prolactin levels were determined in the Pathology Department, University of Leeds, England, using a homologous radioimmune assay (Knight et al., 1977).

The animals were killed at the end of the 13-week dosing period, approximately 24 h after the last treatment, by an overdose of Nembutal. They were subsequently dissected and examined for any gross abnormality.

The pituitary glands were fixed immediately in formaline sublimate (eight volumes of a saturated aqueous HgCl₂ solution and two volumes of formaline [40% formaldehyde]) for 24 h. They were washed in water then in 70% alcohol each for 24 h, then processed following the usual routine technique using toluene as the clearing agent. Longitudinal paraffin sections, not more than 5 μm thick were made in series, and every fifth section was used. A total of ten serial section per hypophysis, representing different levels throughout the whole pituitary were stained, using the modified tetrachrome staining technique (Attia, 1978) and used for microscopical examination. The prolactin (PRL), somatotrophin (STH), adrenocorticotrophin (ACTH) and gonadotrophin-producing cells were differentiated by the staining properties of their cytoplasm and variations in their morphological characteristics were studied in detail. Differential cell counts were made using a projecting microscope at a magnification of 510 × in 50 fields per section, representing the cranial, caudal and central parts of the anterior pituitary (AP). In this way, the mean number of each cell type present in the ten serial sections was calculated and expressed as the relative percentage of cells present in the AP for individual cases. The results are listed in Table 1. The nuclear, cytoplasmic and cell volumes were determined (using the same apparatus for measurements), in 200 of each cell type from the three parts of the adenohypophysis in the serial