Regeneration of the thymus in old male rats treated with a stable analogue of LHRH

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ABSTRACT

It has been shown that the thymus can be regenerated in intact old rats by implanting s.c. a stable analogue of LHRH. Old male rats were given s.c. implants of osmotic pumps containing a solution in citrate buffer of the analogue which was given at a rate of 1 μg/h for 28 days. Some animals were given pumps containing buffer alone, and another group of rats was orchidectomized. The animals were killed after 28 days and the tissues weighed and taken for histology. Serum testosterone was measured by radioimmunoassay.

Sham-treated rats had small fatty thymuses, which were poorly organized with a very narrow band of cortex. Animals treated with the analogue of LHRH and those which had been orchidectomized had relatively large thymuses which were multi-lobed in drug-treated rats, and atrophied accessory sex organs. The testes were grossly atrophied in analogue-treated rats. Histologically, the thymus looked healthy, having a wide, thymocyte-filled cortex and a clearly defined corticomedullary junction. Serum testosterone concentrations were similar in orchidectomized and analogue-treated rats.

It is concluded that it is possible to regenerate the thymus in old rats treated with an analogue of LHRH, but the effect is accompanied by chemical castration. It is also clear that the old pituitary gland is susceptible to the desensitizing action of an LHRH analogue.


INTRODUCTION

Orchidectomy has profound involutional effects on the accessory sex organs of the rat and many other species, and also affects other tissues. For example, it has been known since before the turn of the century that the thymus is actually increased in size after castration (Calzolari, 1898; Henderson, 1904), and it is assumed that the involutional effects on the accessory sex organs and the trophic effects on the thymus are most probably due to the removal of the sex hormones (for review see Grossman, 1985).

There is also much evidence that orchidectomy is associated with an increased immune response to antigen challenge, and that this increase is reduced or prevented by replacement with sex hormones (e.g. Graff, Lappe & Snell, 1969; Castro, 1974; Franks, Perkins & Bishop, 1974; Roubinian, Talal, Greenspan et al. 1978).

We have studied the effects of orchidectomy and replacement with steroid hormones in old (12- to 15-month-old) rats, and found that the thymus, which had virtually disappeared in 18-month-old rats, was greatly restored after orchidectomy (Fitzpatrick, Kendall, Wheeler et al. 1985). In these animals, the total white cell count was raised and in subsequent experiments (F. T. A. Fitzpatrick & B. D. Greenstein, unpublished observations) we have established that the increase was due to lymphocytosis. This raises the possibility that the regenerated thymus might once more make an active contribution to the immune system in old animals. The regeneration of the thymus after orchidectomy of both young adult and old rats was inhibited by implants of testosterone which restored the accessory sex organs (Greenstein, Fitzpatrick, Adcock et al. 1986). Testosterone was, however, unable to restore the accessory sex organs completely in old rats after a
prolonged period of castration (Greenstein et al. 1986).

As a result of our earlier findings, we suggested that thymic regeneration without surgical castration might be accomplished chemically. To address this problem, we attempted to regenerate the thymus using an analogue of luteininizing hormone-releasing hormone (LHRH). The pituitary gland is desensitized to LHRH when continuously exposed to potent analogues of the hypothalamic hormone, and this results in a reversible inhibition of testicular steroidogenesis and spermatogenesis (Linde, Doelle, Alexander et al. 1981).

**MATERIALS AND METHODS**

Male Wistar CSE rats were bred and maintained in the Animal House of the United Medical and Dental Schools of Guy’s and St Thomas’s Hospitals, St Thomas’s Campus. The animals were kept four or five to a cage under conditions of controlled lighting and heating (lights on 08.00–22.00 h; 21–23 °C), and allowed 100 g of Spratt’s Laboratory Diet no. 1 daily per cage. The rats weighed 500–600 g, and ranged from 12 to 15 months of age. We have found that if allowed unlimited access to food the old rats became ill, developed swollen feet, cutaneous tumours and invariably died at a weight of about 700 g.

There were three groups of rats in this study. One group of five rats was orchidectomized bilaterally by the scrotal route under pentobarbitone anaesthesia (Sagatal; May & Baker Ltd, Dagenham, Essex; 40 mg/kg i.p.). Two other groups of non-castrated rats, with eight rats in each group, were used for studies of the action of the drug. Each animal in one group received an s.c. implant under ether anaesthesia of an Alzet osmotic pump, model 2ML 4 (Alza Research, Palo Alto, CA, U.S.A.). The analogue used was ICI 118630 (Zoladex), made by ICI plc, Macclesfield, Cheshire. After continuous exposure to a daily dose of 50 µg, the testes atrophied and the animals showed the classical symptoms of orchidectomy at autopsy (Furr & Hutchinson, 1985). The analogue is more potent than the natural peptide in this respect (Dutta, Furr, Giles et al. 1978). The pump was filled with a solution of Zoladex in citrate buffer (0·1 mol/l; pH 5·0). A release rate of 1 µg Zoladex/h for 28 to 30 days was required, and, according to the maker’s specifications the pumps in this particular batch released 2·5 µl each hour. Therefore a solution containing 400 µg Zoladex/ml was used. The other group was given pumps filled with citrate buffer alone. After 28 days, the animals were deeply anaesthetized with ether and the thorax exposed. Blood was taken by cardiac puncture into heparinized vials and plasma prepared for measurement of testosterone. The thymus was photographed and the thymus, ventral prostate and seminal vesicle glands, spleen and testes were removed and weighed after killing the animal by cervical dislocation. The tissues were prepared for histology by fixation in formol acetic alcohol. Radioimmunoassay of testosterone was as described previously (Greenstein et al. 1986). Statistical tests were carried out using a one-way analysis of variance and the unpaired Student’s t-test where appropriate.

**RESULTS**

Orchidectomy reduced the weight of the accessory sex organs dramatically (Text-fig. 1). The weight of the prostate gland was reduced by approximately two-thirds, and the seminal vesicles by half (P<0·001). The LHRH analogue was significantly (P<0·001) less potent than orchidectomy in reducing prostate weight at the dose used, but was equipotent in the case of the seminal vesicles. The testis was substantially (P<0·001) reduced in weight in animals treated with the LHRH analogue. Histologically, the prostate gland and seminal vesicles appeared normal in old sham-implanted rats in that there was evidence of secretions in the lumina, and the glandular epithelium showed no signs of atrophy (not shown). After treatment with the LHRH analogue the accessory organs were similar in microscopical appearance to those in orchidectomized rats; there was gross atrophy of the glandular epithelium and no sign of secretory activity.

Although the weight of the thymus in intact rats is given (Text-fig. 1), this tissue was fatty when examined histologically (not shown), and the thymus was poorly organized, with a very narrow band of cortex, and an indistinct corticomedullary region. Although there was little visible evidence of thymus tissue (Plate), thymic lymph nodes were large and clearly visible. It was almost impossible to distinguish thymus from fatty tissue at the time of extirpation, so a weight for the fatty tissue cannot be given. However, from the histological evidence, it is estimated that at least two-thirds of the tissue removed was fat in buffer-treated rats.

After orchidectomy or treatment with the LHRH analogue, the thymus was greatly (P<0·001) increased in size, although the LHRH analogue produced a significantly (P<0·001) larger thymus than that observed after orchidectomy. In gross appearance, the thymus produced in analogue-treated animals was unusual in that it was multi-lobed (Plate), and in one case six lobes were counted. Histologically, the thymus appeared normal, in that the cortex was a relatively broad band, densely packed with thymocytes, and well vascularized. The spleen, another lymphoid tissue, appeared to be unaffected either by orchidectomy or the
LHRH analogue. Serum testosterone concentrations were reduced to the same extent in orchidectomized and analogue-treated rats (Text-fig. 2).

**DISCUSSION**

In the present series of experiments our earlier observation that the thymus of old rats is regenerated after orchidectomy (Fitzpatrick et al. 1985; Greenstein et al. 1986) has been confirmed. Furthermore, regeneration of the thymus without surgery has been produced, using an analogue of LHRH. The analogue used was even more potent than orchidectomy in this respect, although not as potent as orchidectomy in reducing the weight of the prostate gland. Since the analogue reduced testosterone concentrations to levels measured in orchidectomized rats, the effect could be explained simply in terms of the removal of testosterone. It should be appreciated, also, that the pituitary gland of old rats is still responsive to the desensitizing action of LHRH. Our earlier observation that the accessory sex organs in old rats atrophy at a slower rate than those of younger animals after orchidectomy (Greenstein et al. 1986) has been confirmed.

It is possible that the analogue was also acting directly on the thymus or through an action on the pituitary gland. There is much evidence that pituitary hormones act directly on the thymus (Denckla, 1978; Comsa, Leonhardt & Ozminski, 1979; Deschaux, Ardail & Guibert, 1981). LHRH-like factors have been found in many peripheral tissues, including rat testis (Bhasin, Heber, Peterson & Swerdloff, 1983), the rat ovary (Ying, Ling, Bohlen et al. 1981) and the placenta (Khodr & Siler-Khodr, 1980), and it has been suggested that this factor may mediate the release of human chorionic gonadotrophin (hCG) (Haning, Choi, Kiggens et al. 1982).

The trophic effect of the LHRH analogue on the thymus which was observed in the present study raises the important question of the consequences for the immune system. There is evidence that castration significantly increases the rate of graft rejection in many species including the rat, and the rate of rejection is

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**TEXT-Figure 1.** Wet tissue weights of old intact (I) male rats, or of rats orchidectomized (OCX) or treated with the LHRH agonist for 28 days (LHRH-A). Results are shown as the means ± s.e.m. The numbers of animals are given in parentheses.
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REFERENCES


slowed if the animals are treated with oestrogens or progesterone (Bilder, 1976). Injections of luteinizing hormone (LH) or follicle-stimulating hormone (FSH) shortened the graft rejection time in castrated animals treated with oestradiol (Bilder, 1976).

There is, therefore, much evidence that while the action of the LHRH analogue on the thymus may be due to a desensitization of the pituitary gland to hypothalamic LHRH, a direct action on the thymus, or an action through other pituitary hormones cannot be ruled out, the results obtained here also indicate that there is a good case for exploring the actions of LHRH and its analogues on the immune system. If it can be shown that the regeneration of the thymus in old animals leads to an enhanced immune system with improvements in resistance to infection, then it becomes very important to seek a means of regenerating the thymus without surgical intervention, and without side-effects such as chemical orchidectomy. In this study, it has been shown that the first of these aims is possible.


DESCRIPTION OF PLATE

FIGURE 1. Exposed thorax of a 15-month-old normal male rat, showing remnants of thymus and fat above the heart. There is no visible evidence of a lobed organ.
Scale bar = 0.5 cm.

FIGURE 2. Exposed thorax of a 15-month-old rat treated for 28 days with the LHRH analogue Zoladex. The thymus is clearly visible as a multi-lobed organ overlying the heart.
Scale bar = 0.5 cm.