Regulation of humoral immunity in rats 
by pituitary hormones

Istvan Berczi¹, Eva Nagy¹, Kalman Kovacs² 
and Eva Horvath²

Department of Immunology¹, University of Manitoba, Winnipeg, Manitoba, Canada and 
Department of Pathology², St. Michael's Hospital, Toronto, Ontario, Canada

Abstract. Hypophysectomized female Fischer 344 and Wistar-Furth rats had severely impaired primary and secondary antibody responses to sheep red blood cells (SRBC). Mercaptoethanol-sensitive (IgM) and mercaptoethanol-resistant (IgG) antibodies were similarly affected. Titers to E. Coli 055:B5 lipopolysaccharide were also significantly decreased in such animals. The antibody response of hypophysectomized rats could be restored by syngeneic pituitary grafts when placed under the kidney capsule or by prolactin treatment. Growth hormone was less effective in this respect than prolactin. Treatment of normal rats with ACTH suppressed their antibody formation to SRBC. These results indicate that the pituitary gland has the potential to regulate humoral immune responses.

Studies on the influence of hypophysectomy on the various immune reactions have yielded controversial results. Thus, hypophysectomized mice and rats were shown to exhibit a decreased antibody response (Lundin 1960; Enerback et al. 1961; Gisler & Schenkel-Hullinger 1971; Comsa et al. 1974) and no change in antibody production (Nagarada 1954; Kalden et al. 1970; Thrasher et al. 1971; Tyrey & Nalbandov 1972) to various antigens. Similarly, it was reported that hypophysectomy had no effect on allogeneic skin graft survival (Enerback et al. 1961; Dann et al. 1979) whereas in another study, graft survival was prolonged in hypophysectomized rats (Prentice et al. 1976). We showed earlier that hypophysectomized rats are deficient in their antibody production against sheep red blood cells (SRBC), in skin reactivity to dinitrochlorobenzene, in the development of adjuvant arthritis and also in rejecting allogeneic skin grafts (Nagy & Berczi 1978). Here, we present evidence indicating that prolactin is involved in the maintenance of immunocompetence.

Material and Methods

Animals

Female Fischer (F) and Wistar-Furth (W) rats weighing 150–170 g were obtained from Canadian Breeding Farm Laboratories, Ltd., Montreal, Canada and from A.R.S. – Sprague Dawley, Madison, Wisconsin, USA, respectively. All the animals were maintained on a standard diet (Wayne’s Laboratory Blocks, with 6% fat content, Chicago, Illinois, USA) and on water supplied ad libitum.

Surgical procedures

Hypophysectomy was performed by the parapharyngeal approach (Lostroh & Jordan 1955; Tarttelin & Gorski 1972). Sham operation was performed on some animals at the same time. Some of the hypophysectomized animals received syngeneic pituitary glands under the kidney capsule (Lu et al. 1977). The completeness of hypophysectomy and graft acceptance was determined for each animal by autopsy at the end of the experiment.

Histology

The pituitary grafts were fixed in 10% buffered formalin and stained with haematoxylin-phloxine and by the PAS technique. For immunocytologic localization of growth
Hypophysectomy inhibits the production of both 2ME-sensitive and 2ME-resistant antibodies. Hypophysectomized (Hyp-X, Δ) and sham-operated (0) animals received 10⁷ sheep red blood cells (SRBC) ip 14 days after surgery, along with a non-operated control group (●). A portion of each serum sample was treated with 2ME (---) and its haemagglutinating capacity was compared with the original sample as described in Material and Methods. Each point in the figure represents the mean ± SEM of 10 animals. Differences between the control and Hyp-X groups were significant (P < 0.01) from days 6 to 15.

Hormone treatment
Porcine adrenocorticotropic hormone (ACTH; purified cortphin-Sterivet Laboratories, Ltd.) was given in daily doses of 10 and 20 μg, human chorionic gonadotrophin (hCG; Choriogonin-Calbiochem) 20 μg daily, bovine prolactin (Prl; NIAMDD-BPRI.-6) was given in 60, 100 and 200 μg doses daily; bovine growth hormone (GH; NIH-GH-B18) was given in 24 and 120 μg doses daily; and human thyroid stimulating hormone (TSH; Thyrotron-Nordic Pharmaceuticals, Ltd.) was applied in 20 μg doses. All hormones were administered sc; GH, Prl and TSH in saline, ACTH and hCG in oil.

Evaluation of the results
All the sera obtained during the various bleeding of an experiment were titrated at the end, using the same batch of reagents. Mean values ± standard errors of the mean are presented in the figures. Data obtained in experimental and control groups for the same day were compared, using Student's t-test in order to determine the significance of the differences.

Induction of antibody response
SRBC were washed with phosphate buffered saline (PBS) at pH 7.2, 3 times, and 10⁷ cells were injected ip to each animal in 1 ml of PBS. The rats were bled from the tail veins at the time of injection and on every third day afterwards. The serum samples were individually stored at -20°C from each animal and titrated by haemagglutination (Takatsy 1955) at the end of the experiments. Some of the sera were treated with 0.1 ml 2-mercaptoethanol (2ME) (Froese 1971) in order to destroy the haemagglutinating capacity of IgM antibodies. The haemagglutinating titer of such treated sera was then compared with the non-treated portion of the same samples. Thymus-independent antibody response was induced against E. Coli 055.B5 lipopolysaccharide (LPS) (Difco) (Treibar & Lapp 1978). Syngeneic rat red blood cells (RRBC) were collected by cardiac puncture with heparin (25 units/ml), were washed three times in PBS and then incubated with LPS (1 mg/ml of PBS) at 37°C for 45 min. The LPS solution used for coating RRBC was heated at 100°C for 1 h prior to use. The coated RRBC were washed again and 8 × 10⁸ cells were injected ip to each animal. The animals were sampled as previously described and the sera titrated with LPS coated RRBC by haemagglutination.
Restoration of the antibody response by pituitary transplant. ● non-operated, ○ sham-operated, ▼ hypophysectomy, ■ hypophysectomy + pituitary transplant. Syngeneic pituitary glands were transplanted under the kidney capsule one week after hypophysectomy. Two weeks after hypophysectomy all the operated animals and the controls were given $10^7$ SRBC ip. Each point shows the mean ± SEM of 10 animals. Non-operated and sham-operated animals compared to Hyp-X graft recipients by t-test: $P < 0.01$ on days 12, 18 and 21, but not on day 15. Hyp-X animals compared to Hyp-X graft recipients: $P < 0.01$ on days 15, 18 and 21.

**Fig. 3.**

Results

Fig. 1 summarizes the results of two separate experiments (one on W and one on F animals), each curve being constructed from titers measured in 10 experimental animals. A portion of each serum sample was treated with 2ME. The results indicate that most of the haemagglutinating antibodies in control animals were 2ME-sensitive (presumably IgM) on day 6 and that the 2ME-resistant titer rose gradually and represented about half of the titer on day 15. The results also suggest that the low titers observed in hypophysectomized animals throughout the experiment were due almost exclusively to IgM antibodies.

**Suppression of the secondary antibody response by hypophysectomy**

In this experiment, the animals received $10^7$ SRBC ip and some of them were hypophysectomized or sham operated two weeks later. A second dose of $10^7$ SRBC was given ip again 14 days after surgery. Fig. 2 summarizes the results of two separate experiments (one on W and one on F animals). Hypophysectomy inhibited significantly the secondary antibody response, although the titers of hypophysectomized animals were as high as the primary response of controls in the previous experiment.

**Reconstitution of hypophysectomized animals by pituitary transplants**

Syngeneic pituitary glands were transplanted under the kidney capsule at one week after hypophysectomy, and two weeks after hypophysectomy, $10^7$ SRBC was injected ip to all operated animals and to controls. Two separate experiments (one on W and one on F animals) are summarized in Fig. 3. Although the antibody response of hypophysectomized graft-recipients reached the level of control groups only on day 15, it was significantly higher than that of hypophysectomized controls on days 15, 18 and 21. In further experiments, each hypophysectomized animal received two syngeneic pituitary grafts. In these experiments, the degree of reconstitution did not improve significantly in comparison with the data presented in Fig. 3.

**Depressed anti-LPS response in hypophysectomized animals**

Two weeks after hypophysectomy and one week after pituitary transplant, the operated groups of animals and controls all received $8 \times 10^8$ syngeneic red blood cells that were coated with LPS. The results of two experiments (one on W and one on F animals) summarized in Fig. 4a, show that hypophysectomized rats responded to some extent to LPS. In these experiments, the response of hypophysectomized animals could not be improved significantly by syngeneic pituitary transplants. However, in some other experiments, significant improvement occurred. Titration of the serum samples with syngeneic red blood cells that were not coated with LPS revealed a weak but well-detectable auto-antibody response (Fig. 4b) which could be removed by absorption with washed rat red blood cells.

**Histology of pituitary grafts**

The histologic findings in the grafts were similar to those described earlier (Kovacs 1961; Graf et al. 1977). The grafts seemed to be well vascularized but in the central portions scar tissue of various sizes was noted (Fig. 5a). It consisted of condensed connective tissues with collagen fibers and a few scattered lymphocytes, plasma cells and histiocytes.
Anti-LPS and autoantibody response in hypophysectomized rats. ● control, ○ sham-operated, △ hypophysectomy, ■ hypophysectomy + pituitary transplant. Two weeks after hypophysectomy and one week after pituitary grafting, the animals and controls were given $8 \times 10^8$ syngeneic red blood cells (RRBC) ip that were coated with LPS. In Fig. 4a, the titers against LPS-coated RRBC are shown, while in Fig. 4b titers obtained with uncoated RRBC are plotted. The anti-LPS titers were significantly lower ($P < 0.01$) in Hyp-X than in control animals on days 6, 9 and 12. There was no significant difference between the Hyp-X group and the Hyp-X pituitary graft recipient group throughout the experiment.

In some cases, mononuclear cell infiltration was more pronounced and foci of mononuclear cells were apparent in several areas of the grafts. The size of the peripheral surviving portion varied from rat to rat. In general, it was substantial, but it was always smaller than the intrasellar adenohypophysial of the untreated rat.

In the surviving portion, the most prominent histologic finding was the profound reduction in the number of recognizable acidophil and basophil cells. Chromophobocytes, degranulated adenohypophysial cells predominated. In some areas, a few acidophils were revealed. Occasionally a few adenohypophysial cells contained small PAS positive cytoplasmic granules.

The immunoperoxidase technique revealed a conspicuous decrease in the number of immunostainable growth hormone cells. Cells showing positive immunostaining for prolactin were numerous (Fig. 5b).

Reconstitution of hypophysectomized rats by Prl treatment

The results of two experiments are shown in Fig. 6. In the first experiment (Fig. 6a) treatment with 200 μg of Prl daily for 10 days restored the immune response of hypophysectomized animals to SRBC to a great extent, while treatment with 24 μg of GH...

a) Two week after hypophysectomy F rats were given 10⁷ SRBC ip and 200 µg of prolactin (Prl) or 24 µg of growth hormone (GH) was given sc daily for 10 days to the respective groups. Each point in the figure represents the mean ± SEM of 5 animals. Titers in the control group were significantly different (P < 0.02) from titers of Hyp-X + GH group, but no significant differences were obtained between control and Hyp-X + Prl. Titers of the Hyp-X group were significantly lower than those of Hyp-X + GH on days 6, 12 and 15 (P < 0.02) and that of Hyp-X + Prl group on days 6, 9 (P < 0.01), 12 (P < 0.02) and 15 (P < 0.01).

b) This experiment was carried out also on F animals according to an identical schedule with the exception that the dose of Prl was lowered 40 µg/day and that of GH was raised to 120 µg/day. Combined application of Prl (100 µg/day) and GH (60 µg/day) as well as of ACTH + hCG + TSH (20 µg/day for each hormone) was also done on additional groups. Titers of Hyp-X + Prl and Hyp-X + Prl + GH groups were significantly higher (P < 0.01) than that of the control group only on day 6 and there was no significant difference at any other points of measurement. Titers of all other groups were significantly lower than control titers from day 6—15 (P < 0.02) — 0.01), except for Hyp-X + GH, which was not significantly different on days 6 and 9.

The effect of ACTH treatment on the antibody response of normal and hypophysectomized rats. ● control, ○ control + ACTH, ▲ Hyp-X, Δ Hyp-X + ACTH.

a) Groups of 5 control and Hyp-X (starting 2 weeks after operation) F rats were given sc 10 µg ACTH daily until the termination of this experiment. SRBC were injected ip 7 days after the commencement of hormone treatment. Titers of all treated groups were significantly lower than control from day 6 to 15 (P < 0.01). Titers of non-operated ACTH treated animals were significantly higher than titers of Hyp-X and Hyp-X + ACTH from days 6—15 (P < 0.01). No significant difference was found between Hyp-X and Hyp-X + ACTH.

b) This experiment was carried out identically to a) except that 20 µg ACTH was given daily to the respective group of animals. Here again, titers of all treated groups were significantly lower than control from day 6 to 15 (P < 0.01). Titers of non-operated ACTH treated animals (P < 0.01—0.02) and significant differences occurred between the Hyp-X and Hyp-X + ACTH groups on days 6 (P < 0.01) and 15 (P < 0.02).
had little effect. In the second experiment (Fig. 6b), the dose of Prl was lowered to 40 μg/day, while the dose of GH was increased to 120 μg/day. Combined treatments with GH and Prl and with ACTH, hCG and TSH were also given to two additional groups. Again, only Prl restored the antibody response of hypophysectomized animals, while GH was virtually ineffective. ACTH, hCG and TSH did not seem to have any effect.

Treatment of hypophysectomized and control animals was carried out with ACTH alone. In this case, hormone treatment was started one week prior to the injection of antigen and continued throughout the experiment. ACTH in 10 μg daily doses lowered significantly the antibody response of control animals, while the response of hypophysectomized animals was unaffected (Fig. 7a). When 20 μg of ACTH was given daily, which is considered by many as a physiological dose) further reduction occurred in the antibody response of control animals. In this case, the weak response of hypophysectomized animals was also reduced somewhat (Fig. 7b). hCG in 20 μg daily doses, did not affect significantly the anti-SRBC response of normal and hypophysectomized rats in 2 experiments.

Discussion

All the hormones used in our experiments are known to be biologically active in rats. However, variability in hormone bioassays due to species differences is not uncommon (Fleischer & Schwartz 1975). These examples dictate caution with the interpretation of our results. Nevertheless, our data indicate clearly that pituitary function is necessary for antibody production to a thymus-dependent (SRBC) and also for an optimal response to a thymus-independent (LPS) antigen. The response of hypophysectomized animals to SRBC (but less to LPS) could be improved significantly by pituitary grafts. As it was shown here and also by others (Chen et al. 1970; Lotz & Krause 1976) transplanted pituitaries produce Prl only in significant quantities; while the production of other pituitary hormones is grossly impaired. Indeed, our subsequent experiments (Fig. 6) indicated that Prl is involved in the maintenance of humoral immunocompetence. It seems unlikely that some other hormone would be responsible for this restoring effect, although it cannot be excluded with complete certainty. In this latter case one would have to prove that pituitary grafts produce an as yet unknown hormone in addition to Prl, which is present also in the purified Prl preparation in quantities sufficient for restoration. Up to now there is no evidence suggesting the existence of such hormone(s).

The suppressive effect of ACTH (Fig. 7) substantiates further the well known immunosuppressive effect of corticosteroids (Claman 1975). GH, which was shown by others to affect the immune system (Gisler & Schenkel-Hullinger 1971; Comsa et al. 1974; Baroni et al. 1969; Fabris et al. 1971) was less effective than Prl in our experiments. The reason for this is unknown at the present time. In two separate experiments treatment with hCG did not influence significantly the anti-SRBC response of normal or hypophysectomized animals. We did not treat normal and hypophysectomized animals with TSH alone. However, Fabris (1973) showed that rats thyroidectomized in young adult age exhibited an impaired antibody response 45–60 days after operation. Since the thyroid gland accumulates a fair amount of biologically active thyroxine, such impairment should occur even later if the thyroid ceases to function because of hypophysectomy. Thus, the impaired antibody response of hypophysectomized animals 4–5 weeks after operation is not likely to be due to the lack of thyroid function.

Apart from the finding that lactation (Dineen & Kelly 1972; Ngwenya 1976) and Prl treatment (Kelly & Dineen 1973) influences the resistance of rats and mice to certain parasites, there is little indication for the possible role of Prl in immune responses. However, though Prl appears to be present in all vertebrates, in lower animal species no clearly predominant action of this hormone is evident. Osmoregulatory, integumentary, growth, developmental and metabolic functions have been claimed for Prl in lower animals. In mammals and birds the predominant role of the hormone is related to reproductive functions (Nicoll 1980). Since the ability to respond to various antigens by specific immunity is also a vertebrate characteristic (Marchalonis 1976), it may well be that the major function of Prl is immunological. Further experiments are required to substantiate and to elucidate in more detail the role of Prl in immunological reactions.
Acknowledgments

The authors are indebted to Drs. H. G. Friesen, A. H. Sehon and E. Sabbadini (University of Manitoba); B. H. Waksman, (Yale University); P. Gold (McGill University); E. P. Potworowski (Institut Armand Frappier, Montreal); A. Nowotny (University of Pennsylvania); R. T. Prehn (Jackson Laboratories, Bar Harbor, Maine) and J. J. Twomey (Veterans Administration Hospital, Houston, Texas) for their valuable suggestions and criticisms. We are also grateful to Mrs. D. Currie for her excellent technical help and Mrs. R. Thomas for her work on this manuscript. Dr. Eva Nagy is an MRC Fellow of Canada. This work was supported by the Medical Research Council of Canada and by the University of Manitoba Research Foundation.

Purified bovine prolactin and growth hormone were kindly donated by Dr. Salvatore Raiti and rat growth hormone and prolactin antibodies by Dr. A. H. Parlow, through the NIAMDD National Pituitary Agency, Baltimore, Maryland, USA.

References


Received on November 18th, 1980.