

Pituitary Hormones and Contact Sensitivity in Rats

I. BERCZI¹ E. NAGY,¹ S. L. ASA² and K. KOVACS²

¹ Dept. of Immunology, Faculty of Medicine, University of Manitoba, Winnipeg, Manitoba and

² Dept. of Pathology, University of Toronto, St. Michael's Hospital, Toronto, Ontario, Canada

Hypophysectomized (Hypo-X) rats do not develop contact sensitivity to dinitrochlorobenzene (DNCB). Daily treatment with prolactin or growth hormone completely restores the DNCB-reactivity of Hypo-X animals. Treatment of such animals with ACTH, FSH, LH, TSH or HCG has no restoring potential. Treatment with ACTH in addition to prolactin or growth hormone antagonizes restoration of Hypo-X rats. These experiments indicate that the pituitary gland has the potential of regulating contact sensitivity.

Key words: ACTH; contact sensitivity; growth hormone; pituitary hormones; prolactin; rat.

Accepted for publication 8 February 1983

An abundance of circumstantial evidence indicates that hormones do influence immune reactions (1). The immunosuppressive action of corticosteroids, especially when administered in pharmacological doses, is very well established (5). ACTH appears to have a similar effect, although this has been studied to a lesser extent (7). It has also been proposed by several authors that growth hormone is needed for the maturation and function of the immune system (2, 6, 8, 10). The effect of other pituitary hormones on immune reactions has not been studied in great detail.

We have shown previously that humoral, cell-mediated and autoimmune reactions are impaired in Hypo-X rats (17), and that the immunocompetence of Hypo-X animals could be restored by syngeneic pituitary grafts or by daily treatment with prolactin (3, 4, 18). In this paper we present studies on the role of various pituitary hormones in the development of contact sensitivity to dinitrochlorobenzene (DNCB) in rats. The results indicate that prolactin and growth hormone maintain contact reactivity, whereas ACTH has a suppressive effect.

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, Quebec, Canada, and from A.R.S.-Sprague Dawley, Madison, Wisconsin, U.S.A., respectively. The animals were maintained on a standard diet (Wayne's Laboratory Blocks, with 6% fat content, Chicago, Illinois, U.S.A.) and on water supplied *ad libitum*.

Surgical procedures

Hypophysectomy was performed by the parapharyngeal approach (15, 21), 3 weeks prior to sensitization. The completeness of hypophysectomy was determined for each animal by autopsy at the end of the experiment.

Immunization. Contact dermatitis was induced by DNCB dissolved in acetone at 200 mg/ml concentration. Twenty μ l of this solution were applied to an area measuring approximately 1 cm² on the shaved dorsal skin. The diameters of

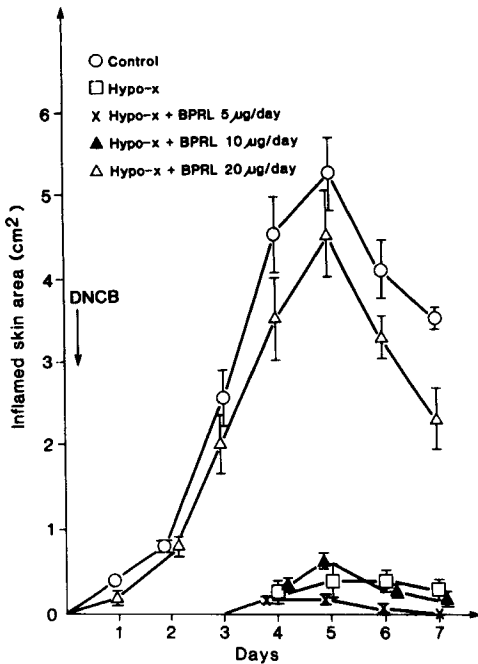


Fig. 1. The reconstitution of DNCB contact sensitivity by prolactin in Hypo-X rats is dose-dependent. Female Wistar Furth (W) rats were hypophysectomized (Hypo-X) and groups of 5 Hypo-X animals along with 5 normal controls were challenged with DNCB 3 weeks later. Treatment with bovine prolactin in 5, 10 and 20 μg daily doses s.c. was initiated on the day of DNCB challenge and maintained for 5 days.

Statistics: Control group was significantly different ($P < 0.01$) from Hypo-X, Hypo-X + 5 or 10 μg PRL throughout days 1–7; and from Hypo-X + 20 μg PRL on days 4, 6, and 7. The group treated with 20 $\mu\text{g}/\text{day}$ of PRL responded significantly better ($P < 0.01$) than Hypo-X on days 1–7. Other PRL-treated groups were not significantly different from Hypo-X. Among the PRL-treated groups, only the one receiving 20 $\mu\text{g}/\text{day}$ was different from the other two ($P < 0.01$) on days 1–7.

the inflamed skin lesions were measured daily in two diagonal directions and the mean affected skin areas $\pm$ SE were calculated for each group. Data obtained in experimental and control groups were compared by the Student's *t* test.

Histology. DNCB-treated skin areas were excised from some animals in control and treated groups on day 6 and fixed with 10% buffered formalin. The specimens were then embedded in paraffin, 4–6 μm thick sections were made, which were stained with hematoxylin and eosin.

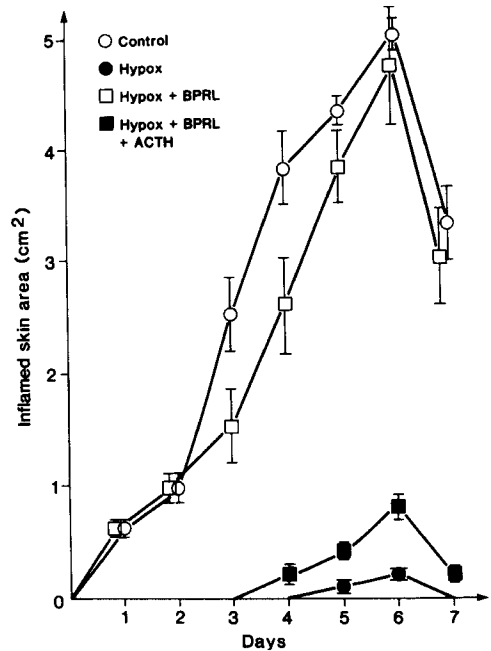


Fig. 2. The antagonistic effect of prolactin and ACTH on DNCB contact sensitivity. Groups of 5 female W rats were Hypo-X, challenged with DNCB and treated with hormones according to the time schedule described for Fig. 1. Both prolactin and ACTH were given s.c. at 40 μg daily doses for 5 days commencing on the day of sensitization.

Statistics: Control group was significantly different ($P < 0.01$) from Hypo-X, Hypo-X + BPRL + ACTH on days 1–7, from Hypo-X + BPRL on days 3–4 ($P < 0.01$) and on day 5 ($P < 0.02$). Hypo-X compared to Hypo-X + BPRL: $P < 0.01$ on days 1–7; Hypo-X compared to Hypo-X + BPRL + ACTH: $P < 0.01$ on days 4–7; Hypo-X + BPRL compared to Hypo-X + BPRL + ACTH: $P < 0.01$ on days 1–7.

Coded slides were evaluated independently by two qualified pathologists (S.A. and K.K.).

Hormone and drug treatment. Porcine adrenocorticotrophic hormone (ACTH) was purchased from Sterivet Laboratories, Bolton, Ontario, Canada; human thyroid stimulating hormone (TSH, Thyrotrol[®]) from Nordic Pharmaceuticals, Ltd., Laval, Quebec, Canada; and human chorionic gonadotropin (HCG, Choriogonin[®]) from Calbiochem. Bovine prolactin (BPRL, NIAMDD-BPRL-6); bovine growth hormone (BGH, NIH-GH-B18); bovine luteinizing hormone (BLH, NIAMDD-bLH-4) and rat follicle stimulating hormone (RFSH, NIADDK-rat

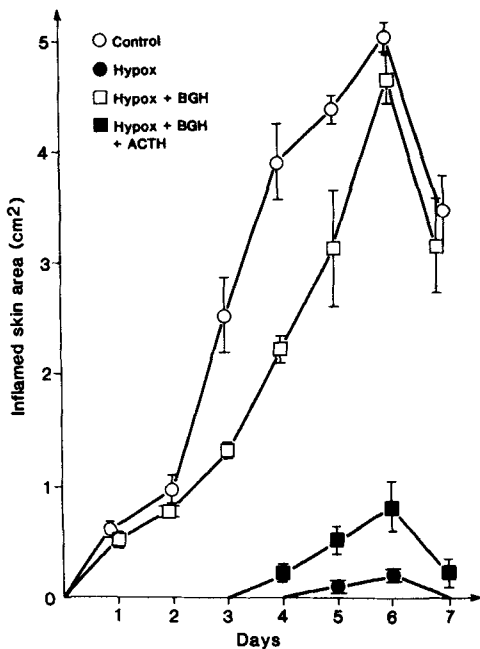


Fig. 3. The antagonistic effect of growth hormone and ACTH on DNCB contact sensitivity. Groups of 5 female W rats were used. For further details, please see legend to Fig. 1.

Statistics: Control group was significantly different ($P < 0.01$) from Hypo-X, Hypo-X + BGH + ACTH on days 1-7; and from Hypo-X + BGH on days 3-5. Hypo-X compared to Hypo-X + BGH: $P < 0.01$ on days 1-7; Hypo-X compared to Hypo-X + BGH + ACTH: $P < 0.01$ on days 4-6; Hypo-X + BGH compared to Hypo-X + BGH + ACTH: $P < 0.01$ on days 1-7.

FSH-B2) were the generous gift of Dr. Salvatore Raiti of the National Pituitary Agency, Baltimore, Maryland, U.S.A. ACTH and HCG were given in oil as supplied by the manufacturer, and the remaining hormones in saline. All hormones were administered subcutaneously in 40 μ g daily doses unless otherwise indicated.

RESULTS

Fig. 1 demonstrates that normal animals developed an inflammatory response to DNCB painting over an average area of 5 cm², whereas the response of Hypo-X rats was less than 1 cm². Treatment of Hypo-X rats with bovine prolactin fully restored their capacity to develop contact sensitivity when at least 20 μ g were given daily.

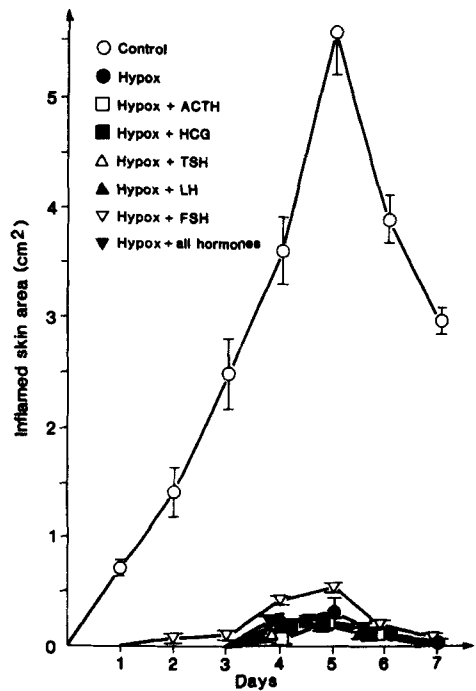


Fig. 4. ACTH, FSH, LH, TSH or HCG do not reconstitute Hypo-X rats for DNCB contact sensitivity. This experiment was performed on groups of 5 W female rats in a similar design to that described in the legend to Fig. 1. All hormones were given at 40 μ g daily s.c. doses for 5 days commencing on the day of DNCB challenge.

Statistics: Control compared to Hypo-X or Hypo-X + hormone treatment: $P < 0.01$ for days 1-7. Hypo-X compared to Hypo-X + FSH: $P < 0.01$ on days 4 and 5. None of the other hormone treated groups were significantly different from Hypo-X or from each other.

Lesser doses showed little effect. When ACTH was administered jointly with BPRL to Hypo-X rats, these two pituitary hormones proved to be antagonistic as shown in Fig. 2. The data presented in Fig. 3 demonstrate that BGH was just as effective in restoring contact sensitivity in Hypo-X animals as was BPRL. Again, ACTH significantly inhibited the restoration achieved by BGH. Further experiments revealed that ACTH, FSH, LH, TSH or HCG did not have the capacity to normalize DNCB contact sensitivity in Hypo-X rats (Fig. 4).

These experiments were also evaluated by light microscopy as shown in Fig. 5. The histologic changes after DNCB treatment in the skin

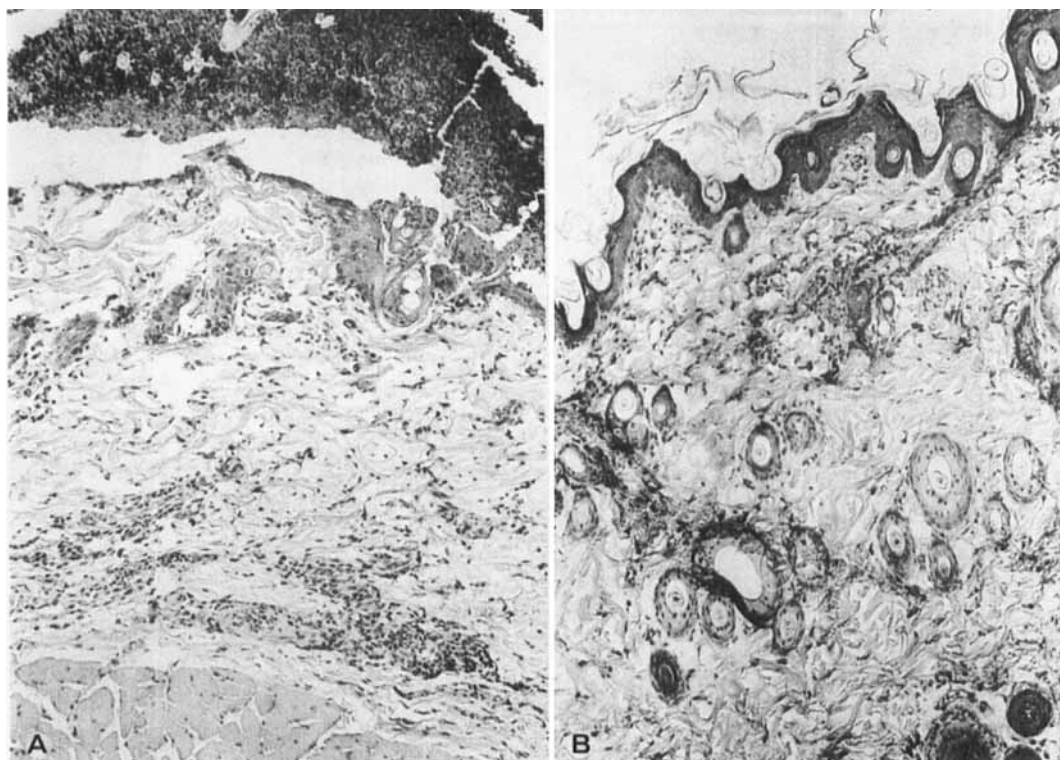


Fig. 5. Histology of DNCB skin reactions in normal and in Hypo-X rats.

(A) Photomicrograph showing intense reactions to DNCB in skin of a nonhypophysectomized control rat; an ulcer is covered by necrotic debris, fibrin, polymorphonuclear and mononuclear leukocytes. The dermis is edematous and there is perivascular infiltration by lymphocytes and macrophages (hematoxylin-eosin stain; original magnification $\times 100$).

(B) Photomicrograph showing skin of Hypo-X rat with slight dermal edema, minimal inflammation and no ulceration (hematoxylin-eosin stain; original magnification $\times 100$).

of the nonhypophysectomized, untreated rats were identical to those described by Willoughby et al. (23). The most striking finding was a large ulcer covered by an amorphous crust consisting of necrotic debris, fibrin, polymorphonuclear leukocytes and mononuclear inflammatory cells. The dermis was edematous and showed multiple foci of inflammation, predominantly perivascular in location and composed mainly of lymphocytes and macrophages. The lesions were minimal in the skins of hypophysectomized untreated rats. No ulceration was evident; edema of the dermis and inflammatory cell infiltration were slight.

Both GH and PRL restored the reactivity of the Hypo-X rats to DNCB. In the skins of these rats, ulceration, dermal edema and inflamma-

tory cell infiltration were identified. Simultaneous administration of ACTH reduced the severity of lesions in Hypo-X, GH or PRL-treated rats.

DISCUSSION

The results presented in this paper demonstrate that of the six pituitary hormones tested, only prolactin and growth hormone had the capacity to restore DNCB contact sensitivity in Hypo-X rats. In earlier experiments, we studied the influence of ACTH and HCG on the immune reactivity of normal rats (3). ACTH suppressed antibody formation, which is in agreement with the findings of other investigators (7). HCG had no major influence on

antibody response when injected into normal animals. We did not treat normal animals with TSH alone. Fabris (9) showed that thyroidectomized young adult rats exhibited an impaired antibody response 45–60 days after operation. Thus, chronic thyroid deficiency seems to cause an impaired immune response. However, TSH treatment of Hypo-X animals had no effect in our experiments.

Whereas it is widely assumed that ACTH affects immune responses through the stimulation of adrenal corticosteroids, which in turn act on lymphocytes (5), the mechanism of action of growth hormone and prolactin is virtually unknown. Human lymphoid cell lines have been shown to have surface receptors for growth hormone and some growth stimulation of both normal and leukemic lymphocytes has been observed under the influence of growth and lactogenic hormones (13, 14, 16). A rat lymphoma cell line which is dependent for growth on lactogenic hormones, has also been described (11, 20). However, there is virtually no evidence for the presence of growth hormone and prolactin receptors in normal lymphoid tissue. It is conceivable that such receptors are absent, or are present only at a very low density on the surface of resting lymphocytes. In this case, the situation would be analogous to that of the insulin receptor (12). An alternative possibility is that prolactin and growth hormone act indirectly on the lymphoid system. It is well established that growth hormone stimulates the production of somatomedins by the liver, which in turn act on various other target tissues (19). The presence of somatomedin receptors has already been described on human lymphocytes (22). Further experiments are required to clarify these possibilities.

ACKNOWLEDGEMENTS

The authors are indebted to Drs. H. G. Friesen, A. H. Sehon and E. R. Sabbadini for their valuable suggestions and criticisms. This work was supported by the Medical Research Council (MRC) of Canada. Dr. Asa is a Fellow of the MRC. Dr. Nagy was an MRC Fellow during the performance of these studies. Purified growth hormone, prolactin, follicle stimulating hormone and luteinizing hormone were kindly

donated by Dr. Salvatore Raiti through the NIAMDD National Pituitary Agency, Baltimore, Maryland, U.S.A.

REFERENCES

1. Ahlqvist, J.: Endocrine influences on lymphatic organs, immune responses, inflammation and autoimmunity. Almquist & Wiksell, Stockholm, 1976.
2. Baroni, C. D., Fabris, N. & Bertoli, G.: Effects of hormones on development and function of lymphoid tissue. *Immunology* 17, 303–314, 1969.
3. Berczi, I., Nagy, E., Kovacs, K. & Horvath, E.: Regulation of humoral immunity in rats by pituitary hormones. *Acta Endocrinol.* 98, 506–513, 1981.
4. Berczi, I. & Nagy, E.: A possible role of prolactin in adjuvant arthritis. *Arthritis Rheum.* 25, 591–594, 1982.
5. Claman, H. N.: How corticosteroids work. *J. Allergy Clin. Immunol.* 55, 145–151, 1975.
6. Comsa, J., Schwarz, J. A. & Neu, H.: Interaction between thymic hormone and hypophyseal growth hormone on production of precipitating antibodies in the rat. *Immunol. Commun.* 3, 11–18, 1974.
7. Comsa, J. & Leonhardt, H.: Influence of the thymus-corticotropin-growth hormone interaction of skin allografts in the rat. *Ann. N.Y. Acad. Sci.* 249, 387–401, 1975.
8. Fabris, N., Pierpaoli, W. & Sorkin, E.: Hormones and the immunological capacity. IV. Restorative effect of developmental hormones or of lymphocytes on the immunodeficiency syndrome of dwarf mouse. *Clin. Exp. Immunol.* 9, 227–240, 1971.
9. Fabris, N.: Immunodepression in thyroid-deprived animals. *Clin. Exp. Immunol.* 15, 601–611, 1973.
10. Gisler, R. H. & Schenkel-Hullinger, L.: Hormonal regulation of the immune response. II. Influence of pituitary and adrenal activity on immune responsiveness in vitro. *Cell. Immunol.* 2, 646–657, 1971.
11. Gout, P. W., Beer, C. T. & Noble, R. L.: Prolactin-stimulated growth of cell cultures established from malignant Nb rat lymphomas. *Cancer Res.* 40, 2433–2436, 1980.
12. Helderman, J. H., Reynolds, T. C. & Strom, T. B.: Insulin receptor as a universal marker of activated lymphocytes. *Eur. J. Immunol.* 8, 589–595, 1978.
13. Lesniak, M. A., Roth, J., Gorden, P. & Gavin, J. R., III: Human growth hormone radioreceptor assay using cultured human lymphocytes. *Nature New Biol.* 241, 20–23, 1973.
14. Lesniak, M. A., Gorden, P., Roth, J. & Gavin, J. R., III: Binding of ^{125} I-human growth hormone to specific receptors in human cultured lymphocytes. Characterization of the interaction and a sensitive radioreceptor assay. *J. Biol. Chem.* 249, 1661–1667, 1974.
15. Lostroh, A. J. & Jordan, C. W.: Improved procedure for hypophysectomy of mouse. *Proc. Soc. Exp. Biol. Med.* 90, 267–269, 1955.
16. Mercola, K. E., Cline, M. J. & Golde, W. G.: Growth hormone stimulation of normal and leukemic human

- T-lymphocyte proliferation *in vitro*. *Blood* 58, 337-340, 1981.
17. Nagy, E. & Berczi, I.: Immunodeficiency in hypophysectomized rats. *Acta Endocrinol.* 89, 530-537, 1978.
18. Nagy, E. & Berczi, I.: Prolactin and contact sensitivity. *Allergy* 39, 429-431, 1981.
19. Phillips, L. S. & Vassilopoulou-Sellin, R.: Somatomedins. *N. Engl. J. Med.* 302, 371-380, and 438-446, 1980.
20. Tanaka, T., Shiu, R. P. C., Gout, P. W., Beer, C. T., Noble, R. L. & Friesen, H. G.: A new sensitive and specific bioassay for lactogenic hormones: measurement of prolactin and growth hormone in human serum. *J. Clin. Endocrinol. Metab.* 51, 1058-1063, 1980.
21. Tarttelin, M. G. & Gorski, R. A.: A ventral surgical approach to the anterior tuberal hypothalamus of the rat with specific emphasis on recovery. *Endocrinology* 90, 1128-1132, 1972.
22. Thorsson, A. V. & Hintz, R. L.: Specific ^{125}I -somatomedin receptor on circulating human mononuclear cells. *Biochem. Biophys. Res. Commun.* 74, 1566-1573, 1977.
23. Willoughby, D. A., Walters, M. N. I. & Spector, W. G.: Lymph node permeability factor in the dinitrochlorobenzene skin hypersensitivity reaction in guinea pigs. *Immunology* 8, 578-584, 1965.

Address:

Dr. I. Berczi
Associate Professor
Immunology Department
Faculty of Medicine
University of Manitoba
795 McDermot Avenue
Winnipeg, Manitoba
Canada R3E 0W3