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Virus-Induced Corticosterone in Hypophysectomized Mice: A Possible Lymphoid Adrenal Axis

Abstract. *Infection of hypophysectomized mice with Newcastle disease virus caused a time-dependent increase in corticosterone and interferon production. Prior treatment with dexamethasone completely inhibited the virus-induced elevation in corticosterone concentration, but did not significantly alter the interferon response. Lymphocytes appear to be the most likely source of an adrenocorticotropin-like substance that is responsible for the increased corticosterone, since spleen cells from the virus-infected, but not from control or dexamethasone-treated, hypophysectomized mice showed positive immunofluorescence with antibody to adrenocorticotropin-(1–13 amide). Thus the adrenocorticotropin-like material and interferon appear to be coordinately induced and differentially controlled products of different genes. These findings strongly suggest the existence of a lymphoid-adrenal axis.*

Increases in corticosteroid concentrations are usually considered to be mediated by the pituitary-adrenal axis and to result from increased release of adrenocorticotropin (ACTH) from the pituitary gland (*1*). Recently, we demonstrated in vitro that substances that induce interferon (IFN)-α, such as viruses, cause production of an ACTH-like substance from human lymphocytes (*2*) that is strongly associated with IFN-α. The ACTH-like substance and IFN-α were dissociated by acid (*p*H 2) and were never associated if lymphocytes were induced in the presence of tunicamycin (*2*). These findings strongly suggest that the ACTH-like substance and IFN-α are coordinately induced products of different genes and become associated through a carbohydrate interaction. These findings imply that certain stimuli, which effect lymphocyte production of IFN, should not require the participation of the pituitary gland for an ACTH-mediated increase in corticosteroids. Furthermore, they suggest that lymphocyte production of ACTH in vivo might be dissociable from IFN production. These concepts were tested by determining (i) whether virus-infected hypophysectomized mice would have an increase in ACTH-like activity, thus showing an elevated corticosterone concentration and (ii) whether this putative response is dissociable from IFN production.

Hypophysectomized, female Swiss Webster mice were infected with Newcastle disease virus (NDV) (*3, 4*). These mice showed a time-dependent increase in corticosterone production (Fig. 1A). Peak steroid concentrations occurred at 8 hours after infection and thereafter declined. Serum IFN increased coordinately with corticosterone (data not shown). Thus the production of corticosterone was related to the induction of IFN and, by inference, induction of the ACTH-like substance. Corticosterone concentrations at 8 hours (10 ± 2.6, mean ± standard error, $N = 13$) differed from those at 0 hour (2.76 ± 0.11, $N = 19$) at a probability of $< .01$ (two-tailed Student's *t*-test). Whereas hypophysectomized animals did not respond to a classical "stressor" (cold water) they did respond to ACTH (Fig. 1B). The response to a pharmacologic dose of ACTH was not maximal since there was probably some adrenal atrophy in the 5 days between the hypophysectomy and the experiment (*4*). This suggests that under optimal conditions the adrenal response to virus might be even higher than the 3.5-fold increase that was observed.

The production of ACTH by the pituitary gland, and thus the production of steroid by the adrenal glands, is controlled by negative feedback via adrenal corticosteroids (*1*). The virus-induced increases in corticosterone concentration in hypophysectomized mice appear to be similarly controlled, because prior treatment with the synthetic steroid dexamethasone completely inhibited the increase in corticosterone at 8 hours after NDV infection (Fig. 1B). That lymphocytes are a likely source of an

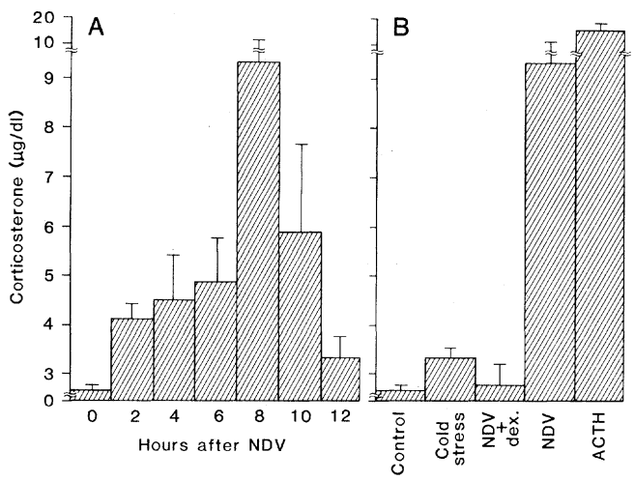

Fig. 1. (A) The kinetics of corticosterone production after NDV infection of hypophysectomized mice. Mice were injected intraperitoneally with 800 hemagglutination (HA) units of NDV in 0.2 ml. At the indicated times, the mice were decapitated and trunk blood was collected for corticosterone determination by radioimmunoassay (*7*). Mouse heads were dissected and examined under a dissecting microscope, and animals with any remnant of the pituitary gland were discarded. The numbers of mice for 0, 2, 4, 6, 8, 10, and 12 hours were 19, 2, 9, 16, 13, 12, and 5, respectively. (B) Dexamethasone (*dex.*) suppression of corticosterone production by NDV-infected hypophysectomized mice. The mice were given free access to water with or without dexamethasone (20 μg/ml) for approximately 24 hours. Control (NDV, $N = 13$) or dexamethasone-treated (NDV + dex, $N = 5$) mice were then injected, as before, with NDV. Eight hours after infection the mice were killed and treated as in (A). Other controls without dexamethasone included: controls ($N = 19$), in which saline (0.2 ml) was injected intraperitoneally and the mice were killed 8 hours later; cold stress ($N = 5$), in which the mice were placed in ice water for 45 seconds and killed 20 minutes later, and ACTH ($N = 9$), in which the mice received an intramuscular injection of 25 μg of Cortrosyn (Organon, Inc., West Orange, New Jersey) in 0.2 ml and were killed 2 hours later. Corticosterone concentrations are expressed as means ± standard error.

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ACTH-like material that causes the virus-induced increases in corticosterone concentration is suggested by our findings that spleen cells from NDV-infected, but not from control or dexamethasone-treated, hypophysectomized mice show positive immunofluorescence with antibody to ACTH-(1–13 amide) (Fig. 2), and that lymphocyte production of the ACTH-like material is controlled both in vivo (Fig. 1B and Fig. 2) and in vitro (*5*) by dexamethasone. However, the concomitant production of extrapituitary ACTH by sources in addition to lymphocytes has not been eliminated.

In contrast, serum IFN levels at 8 hours after NDV infection were similar in both control (10^6 U/ml) and dexamethasone-treated hypophysectomized mice ($10^{5.5}$ U/ml). Thus, whereas dexamethasone suppressed the corticosterone response (Fig. 1B) and spleen cell production of the ACTH-like material (Fig. 2), it did not significantly alter the IFN response. Similar findings have been observed in vitro. Following NDV infection, control and dexamethasone-treated human lymphocytes produced the same amount of IFN whereas only the controls produced the ACTH-like material (*2*). These data show that both in vitro and in vivo the ACTH-like material and IFN are coordinately induced but differentially controlled. Furthermore, they support the idea that these two substances are products of different genes.

These findings strongly suggest that a lymphoid-adrenal axis functions through a lymphocyte-derived substance that may be related to or identical with ACTH. In contrast to the pituitary-adrenal axis, the putative lymphoid-adrenal axis would be stimulated by noncognitive factors recognized not by the central nervous system but by lymphocytes. These might include substances that induce IFN-α, such as viruses, bacteria and their products, and tumor cells. Further, they may be one cause of the well-known increases in steroid concentrations observed during infections. Thus, the immune system may serve a sensory function.

Among clinicians there seems to be a consensus that patients who lack ACTH in the pituitary or who have generalized hypopituitarism have less requirement for cortisol replacement than patients who lack the entire adrenal (*1*). In spite of this, clinical examples of an extrapituitary source of ACTH being stimulated in times of acute stress are rather indefinite. Investigators examining the corticosteroid response of patients with hypopituitarism to stress generally measure the response for only 2 hours after the stress. A response that was mediated through the lymphocytes would therefore be missed. A more appropriate time course for study would be between 2 and 8 hours after stress. Perhaps a more crucial variable is the stimulus for the corticosteroid response. Only those stimuli which effect lymphocyte production of the ACTH-like substance might be expected to cause the response. That humans may have an extra pituitary source of ACTH for stimulation of adrenal secretion is indicated by studies with bacterial polysaccharide, a possible lymphocyte stimulator. Van Wyk *et al.* (*6*) observed significant corticosteroid responses in seven out of eight hypophysectomized patients treated with bacterial polysaccharide (Piromen). The response in one of these subjects peaked at 8 hours. In view of these data from the clinical literature, the experiments described herein indicate the need for further study of the corticosteroid response in the hypophysectomized individual. The present results and previous data (*2*) also provide evidence of intriguing associations between the immune and neuroendocrine systems. Such direct interactions between these two systems may be important for understanding the symptomatology of infectious diseases, tumors, and neuroendocrine disorders. Furthermore, if it could be shown that, as with many other immunologic responses, the lymphocyte production of a neuroendocrine hormone was controlled by the major histocompatibility complex, this would provide a possible etiologic site for putative histocompatibility-linked neuroendocrine diseases.

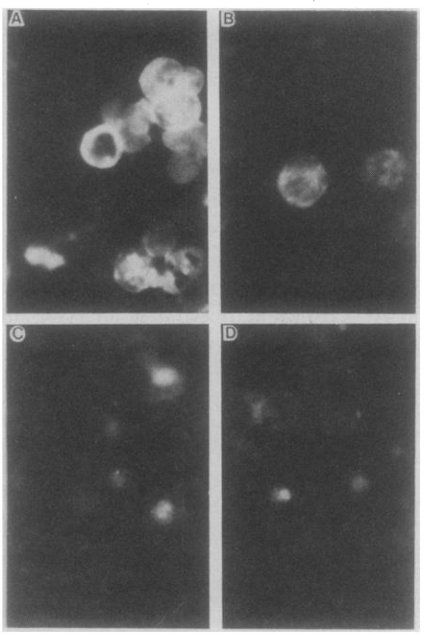

Fig. 2. Immunofluorescent detection of an ACTH-like substance in spleen cells from NDV-infected hypophysectomized mice (*8*). (A) Spleen cells 8 hours after infection stained with antiserum to ACTH-(1–13 amide) or (B) with normal rabbit serum; (C) spleen cells from dexamethasone-treated mice 8 hours after infection, stained with antiserum to ACTH-(1–13 amide); and (D) spleen cells from noninfected mice stained with antiserum to ACTH-(1–13 amide).

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3. Newcastle disease virus was chosen because it replicates very poorly, if at all, in mouse cells. It also effectively induces the lymphocyte-derived ACTH-like substance in vitro (*2*).
4. The hypophysectomized mice (each about 25 g) were obtained from Taconic Farms, Germantown, N.Y. Experiments were performed 5 days after surgery because this was the minimum time for shipment. Each animal was examined post-mortem under a dissecting microscope to assure that the hypophysectomy was complete. Animals with any remnant of the pituitary gland were discarded.
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8. Spleens were removed from hypophysectomized mice, minced, and gently pipetted up and down in phosphate-buffered saline to obtain single-cell suspensions. Cells were fixed on coverslips and stained with a 1:50 dilution of rabbit antiserum to ACTH-(1–13 amide) (Code 1660, Bio-Ria, Brussels, Belgium) or normal rabbit serum by an indirect immunofluorescent technique [D. D. Porter, I. Wimberly, M. Benyesh-Melnick, *J. Am. Med. Assoc.* **208**, 1675 (1969)]. For the specificity of the staining procedure, see (*2*).
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