Perspective

The Regulation of ACTH Secretion by IL-1

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THIS ISSUE OF *SCIENCE* PRESENTS THREE ARTICLES SHOWing that interleukin-1 (IL-1), a polypeptide monokine product of mononuclear phagocytes that mediates a host organism's response to infections or inflammation, can also stimulate the secretion of adrenocorticotropic hormone (ACTH) from the corticotroph cells of the anterior pituitary gland either by acting directly on the normal pituitary cell or by stimulating the release of corticotropin-releasing factor (CRF) from hypothalamic neuroendocrine cells. In the case of CRF neurosecretion, this hypothalamic hormone is released into the portal venous circulation that perfuses the anterior pituitary gland, and, in turn, produces the release of ACTH from its target corticotroph cells. The subsequent role of the ACTH is to stimulate the secretion of endogenous steroid hormones, such as cortisol in the human or corticosterone in the rat, from the cortex of the adrenal gland. These adrenal glucocorticoid hormones can then mediate essential metabolic and immune aspects of the stress response. The importance of the ability of IL-1 to promote ACTH secretion stems from the efficient and dramatic way in which mammalian organisms respond to microbial infections, trauma, inflammatory processes, and any other physical (and probably psychological) stressors. Host responses to microbial, chemical, or traumatic insults take the form of the acute-phase response, which is primarily mediated by IL-1 and includes, but is not limited to, certain catabolic changes for the mobilization of metabolic substrates, the production of specific immune substances by the liver, an increase in the number and immaturity of circulating neutrophils, and fever production (*1*). IL-1 also activates T and B cell function, promotes IL-2 production, and synergizes with lymphokines to enhance natural killer cell activity directed against certain tumor target cells. With such a powerful immune reaction being driven by IL-1, it is necessary for the body to have a mechanism by which this aggressive process can ultimately be reduced so that it does not run amok. Perhaps the most direct and parsimonious system to accomplish this self-regulating goal would be for IL-1 to have an ACTH-stimulating capacity. The secretion of ACTH in response to IL-1 could then stimulate the production and secretion of cortisol or corticosterone, and these glucocorticoid hormones would subsequently act to suppress the further production of IL-1; this latter negative regulatory system has already been demonstrated (*2*). Thus some degree of control could be exerted over these components of the immune response. Adrenal glucocorticoids such as cortisol do, in fact, suppress several aspects of immune function (*3*), and this accounts for their use as immunosuppressants in such procedures as organ transplantation (*4*).

In the three articles being discussed, the three sets of authors agree that IL-1 can in some fashion stimulate ACTH release. However, the site at which IL-1 produces this effect is in dispute among the three groups. Bernton and his colleagues observed that recombinant human IL-1β (Cistron Technology, New Jersey) directly released ACTH from corticotroph cells dispersed from the anterior pituitary glands of female rats at random phases of the estrous cycle. Murine IL-1, which they also tested with cells, was apparently slightly less active. Sapolsky *et al.*, using human IL-1α (supplied by P. Lomedico, Hoffmann–La Roche) and male donor rats as the source of cultured corticotrophs, found no ACTH-releasing activity of IL-1 on pituitary cells prepared in a 3-day culture similar to that used by Bernton *et al.* They also failed to see any effect of murine IL-1 on ACTH release. Berkenbosch *et al.* used IL-1β (supplied by C. A. Dinarello, Boston) and dispersed pituitary cells from female rats at random phases of the estrous cycle. Their cell preparation had been maintained in a 4-day culture. In agreement with the results of Sapolsky and his co-workers, this group observed no effect of IL-1 to stimulate the release of ACTH directly from pituitary cells.

Certain differences among these three investigations might explain their apparent conflicting results regarding a pituitary site of action for IL-1 in the control of ACTH secretion. One interesting difference was the ACTH-releasing potency of the β form of IL-1 in corticotrophs observed by Bernton *et al.* and the lack of this effect for the α form of IL-1 used in the Sapolsky study. Rather than regarding the findings of one group to be correct and the other incorrect, it may be useful to consider the differences in structure between IL-1β and IL-1α. March *et al.* (*5*) showed that the positions of only 70 of 271 amino acids (26%) of human IL-1β are identical to those of human IL-1α and only 80 of 270 amino acids (30%) are significantly identical to those of murine IL-1. By contrast, human IL-1α and murine IL-1 share 167 of 271 (62%) positions. With the knowledge that the amino acid composition of the α and β forms of IL-1 are different, it is less surprising that Bernton's group observed stimulation of ACTH release in vitro with human IL-1β while Sapolsky and coworkers did not detect an effect with human IL-1α. This structural dissimilarity could make IL-1α a poor ligand for a putative IL-1β–specific receptor on pituitary cell membranes. This idea brings up the equally interesting suggestion that IL-1α receptors might predominate at a hypothalamic rather than at a pituitary level. It still must be asked, however, why the murine IL-1 could show some ACTH-releasing activity in the hands of Bernton *et al.* but not in those of the Sapolsky laboratory. A possible explanation may lie in the sex differences between the cell donors. It may be possible that the estrogen milieu of female rats (used by Bernton *et al.*), which of course is less prevalent in males (used by Sapolsky *et al.*), sensitizes the corticotrophs to agents of release just as estrogen can produce the well-characterized sensitization of the gonadotrophs—the luteinizing hormone and follicle-stimulating hormone cells—to the releasing action of luteinizing hormone-releasing hormone (LHRH). Consistent with this hypothesis is the report by Genazzani *et al.* (*6*) that, during the normal menstrual cycle of women, plasma ACTH and cortisol reach their highest levels 1 to 2 days prior to midcycle, the time during which plasma estradiol concentrations reach their highest peak (*7*). From this, then, comes the proposition that the rising estrogen influence immediately prior to the time of the midcycle surge of gonadotropic hormones may also make the corticotrophs increasingly susceptible to the stimulatory action of various ACTH secretagogues. This possibility may also bear on the failure of Berkenbosch *et al.* to observe an induction of ACTH release by human IL-1β from female rat pituitary cells. Since the pituitary cells in the studies of both Bernton *et al.* and Berkenbosch *et al.* were taken from female rats during random times of the estrous cycle (analogous hormonally to the menstrual cycle of the human female), the possibility exists that Bernton *et al.* collected by mere chance a preponderance of cells

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from female rats during the phase of heightened estradiol influence (proestrus) and thus increased cell sensitivity to secretagogues (CRF and interleukin), while the other group had harvested cells predominantly from a group of females not in a period of high estrogen exposure (diestrus) and consequently less responsive to ACTH-stimulating substances. Certainly, this is only speculation and is not possible to ascertain without the availability of vaginal cytology for each animal but, nevertheless, a possibility that is worth a moment's thought. Another explanation for the discordant pituitary findings might be that the different sources, handling, and storage of each laboratory's supply of IL-1 could have variably altered the efficacy of the material they used.

Also provocative is the finding of Bernton *et al.* that human IL-1β at a concentration as low as $10^{-12}M$ will significantly stimulate the release of thyroid-stimulating hormone (TSH), growth hormone (GH), and luteinizing hormone (LH), while inhibiting prolactin (PRL) secretion, from the same pituitary cell preparations used for the examination of interleukin's control of ACTH release. Such broad hormonal effects by IL-1 might cause concern initially about the specific role to be played by this monokine in the regulation of ACTH secretion. However, since it has been shown that one of the major intracellular mechanisms of IL-1 activity is the stimulation of the synthesis of prostaglandins (cyclooxygenase products) in some cell types (*1, 8*) and leukotrienes (lipoxygenase products) in others (*1, 9*), and since both prostaglandins and leukotrienes have been proposed to be endogenous stimulators of the release of LH, ACTH, GH, and TSH from pituitary cells in culture (*10, 11*), the release of multiple hormones by IL-1 seen by Bernton *et al.* is not entirely unexpected. Indeed, such results may even be viewed as a validation of the efficacy of their IL-1 preparation which should, in fact, be able to stimulate these previously defined intracellular mediators of its action and thereby produce the release of all appropriately regulated pituitary hormones. These authors also point out that blockade of the cyclooxygenase pathway with indomethacin in their pituitary cell preparation did not alter the IL-1–induced stimulation of ACTH or LH release, thus redirecting attention to the function of the alternate arachidonic acid metabolic route—the lipoxygenase pathway—being responsible for their release. In support of this hypothesis, Conte *et al.* (*12*) proposed and Hulting *et al.* (*13*) demonstrated that leukotrienes play a direct role in the release of LH from pituitary cells in vitro. The IL-1–stimulated leukotriene mechanism may also operate for the release of other pituitary hormones, just as prostaglandin E_2 has been identified as a direct regulator of the release of almost every anterior lobe hormone (*10*).

The Bernton group also provides convincing data that IL-1 may exert a direct effect on pituitary cell secretion by showing that preincubation of IL-1 with an antiserum generated against this polypeptide (1:1250 dilution) abolishes the IL-1–induced release of ACTH, LH, GH, and TSH, while also preventing the inhibition of PRL secretion by IL-1. Further, they provide evidence that no contaminating substance in their IL-1 preparation could be responsible for the stimulation or inhibition of hormone release seen in their cell cultures and that the IL-1 molecule was not being falsely read as any pituitary-like compound by the antisera they utilized for their hormone radioimmunoassays.

Although the report of Bernton *et al.* does not address the issue of a suprapituitary site of action for IL-1, these authors nevertheless point out that IL-1, whether produced peripherally or centrally, might act at the hypothalamic level to modify the neurosecretion of certain hypothalamic-releasing hormones. The reports from Sapolsky *et al.* and Berkenbosch *et al.* focused on this possibility by examining CRF and ACTH release in response to the action of IL-1 on the hypothalamus in vivo. One of the approaches of the Sapolsky group was to inject human IL-1α intravenously into undisturbed male rats and measure ACTH and corticosterone levels in plasma 10 minutes after IL-1 administration. This treatment elicited a dose-related elevation in plasma ACTH and corticosterone that could be blocked completely by prior intravenous injection of antiserum to CRF. More directly, they also showed that intravenous infusion of IL-1α into anesthetized male rats would stimulate a significant increase in the levels of CRF which they measured directly in the blood of the hypothalamic-hypophyseal portal vessels. These data strongly indicate that IL-1α can exert its control over the adrenocortical axis at the level of the hypothalamic CRF neuron. As mentioned above, however, these investigators failed to detect any ability of IL-1α to alter ACTH secretion from the cultured pituitary cells of male rats. The findings of Berkenbosch *et al.* reinforce those of the Sapolsky study since they too found that systemic injection into male rats of IL-1β (given intraperitoneally) would increase radioimmunoassayable concentrations of plasma ACTH, but that subsequent administration of antiserum to CRF would prevent most, but not all, of the IL-1–induced stimulation of ACTH release. Their interpretation of this residual ACTH response to IL-1 is that the CRF antiserum either failed to neutralize fully all the available circulating CRF or that additional stimulating factors might have been present. While these are plausible explanations, another equally valid interpretation is that the administered IL-1β, in addition to its hypothalamic site of action, was also exerting some direct action on pituitary corticotrophs to cause the two- to threefold release of the residual ACTH. If this were the case, even complete blockade of CRF with the antiserum would not abolish IL-1–stimulated ACTH secretion.

Attempts to reconcile the seemingly incompatible results of these three reports may provide the opportunity to formulate hypotheses to unify these well-supported findings and to form the basis for future investigations into mechanisms by which the immune system may exert a powerful and specific degree of control over the brain's neuroendocrine axes. One action of IL-1 that should be considered in view of its reported ability to increase directly and dramatically the secretion of several pituitary hormones in addition to ACTH, is its mitogenic action on certain cell types of the immune system (*1*). Because it was recently demonstrated that growth hormone–releasing factor (GRF) can stimulate the proliferation of pituitary somatotrophs in vitro (*14*) and because it is proposed, in effect, that IL-1 can function as a "tissue-releasing factor" for corticotrophs (*15*), it would be worthwhile to investigate whether IL-1 can produce the remarkable increases in pituitary secretions seen in vitro by increasing the number, as well as the secretion rates, of these pituitary cell types. If found to be true, then it should be asked whether deranged interleukin production could be implicated in the pathogenesis of certain pituitary tumors.

Of great pertinence to the issues raised here is the fact that astroglial and microglial cells of the brain, which are numerous in the median eminence region of the hypothalamus (*16*), produce IL-1 (*17*). This obviously places them in a favorable anatomic position to modify hypothalamic CRF secretion, as pointed out by Sapolsky *et al.* But these glials cells also have an anatomical component, not discussed in these papers, which can underpin the findings of Bernton *et al.* That is, glial cells project their processes onto the portal capillaries of the median eminence (*16*) through which flows the portal blood that could conceivably deliver the brain-secreted IL-1 directly to the corticotrophs, somatotrophs, thyrotrophs, gonadotrophs, and lactotrophs of the adenohypophysis. Neuroanatomically, then, astroglial IL-1 is situated to function as a hypothalamic releasing or inhibiting hormone and as a potentiator of the action of classically defined releasing hormones on their target cells in the pituitary gland. In addition, IL-1 has already been described

to be equipotent with a combination of CRF and arginine vasopressin in releasing ACTH from the mouse pituitary AtT-20 cell line in vitro (*18*); however, the use of these cells and their responses are considered controversial since they do not reflect the normal state (*19*). Certainly, the levels of IL-1 in portal plasma need to be measured in a variety of physiological situations that alter ACTH and glucocorticoid secretion as well as the secretion of other anterior lobe hormones. Other neural functions of brain IL-1 may also deserve examination.

Finally, the exploration of a sex difference in the production and subsequent action of IL-1α and IL-1β at either the pituitary or hypothalamic level, or both, also seems to be warranted. To reiterate, IL-1 stimulates either the release of CRF or ACTH, or both, directly. This causes the adrenal production and secretion of glucocorticoids, and it is known that glucocorticoids inhibit IL-1 and IL-2 production (*1, 2*). In this fashion, increased glucocorticoid concentrations can control the clonal expansion (driven by IL-1 and IL-2) of committed cells of the immune system that have high affinities for antigens, as explained earlier by Besedovsky *et al.* (*20*). As Besedovsky himself has pointed out (*20*), this regulatory loop may play a role in preventing too vigorous an immune response in the form of development of autoimmune diseases. Since it has been observed that the occurrence of autoimmune diseases such as rheumatoid arthritis and Hashimoto's thyroiditis are more prevalent in women than men (*21*), determination of gender association to the production and action of various interleukin species on the hypothalamic-pituitary-adrenal axis might provide clues to the etiology and treatment of certain autoimmune disorders.

Whatever roles for IL-1 in neuroendocrine regulation may eventually be described, the tentative conclusion that may be assembled from these three studies is that various IL-1 species exert a positive control over ACTH secretion at both the hypothalamic CRF neuron and the ACTH-containing corticotroph, much as glucocorticoids operate at the same two levels to provide negative feedback regulation of adrenocortical function.

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