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CYTOKINES AND THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

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Summary—After administration of the cytokines interleukin 1 (IL1), tumor necrosis factor (TNF), interleukin 2 and interleukin 6 to laboratory animals or humans, plasma levels of glucocorticoids are elevated. This effect is mediated by activation of the hypothalamic–pituitary unit. IL1 and TNF inhibit aldosterone production by rat adrenocortical cells *in vitro* and stimulate renin release by rat renal cortical cells. Administration of IL1 or TNF in rats suppresses hypothalamic–pituitary–thyroid function, whereas IL1 acts at the level of the brain and the gonads to interfere with gonadotropin and sex steroid secretion.

During stimulation of the immune system (e.g. during infectious diseases), peculiar alterations in hormone secretion occur (hypercortisolism, hyperreninemic hypoaldosteronism, euthyroid sick syndrome, hypogonadism). The role of cytokines in these alterations remains to be established.

INTRODUCTION

In 1975 Besedovsky and co-workers [1] observed that in rats and mice at about the time of the peak of the immune response to sheep or horse red blood cells serum corticosterone levels were increased 2–3-fold. In a later study [2] these researchers showed that in mice inoculated with Newcastle disease virus blood levels of adrenocorticotropin (ACTH) and corticosterone increased remarkably. A similar response occurred when the animals were injected with supernatants derived from cocultures of Newcastle disease virus preparations and either human peripheral blood leucocytes or mouse spleen cells, indicating that these immune cells might be stimulated by the virus to produce one or more factors which influence the pituitary–adrenal axis. There is now a considerable body of evidence that these factors belong to the cytokine family [3]. It has been proposed that interleukin 1 (IL1) plays a pivotal role in the activation of the pituitary–adrenal axis during stimulation of the immune system [4, 5]. However, recent studies suggest that other cytokines [interleukin 2 (IL2), interleukin 6 (IL6) and tumor necrosis factor (TNF)] might also be involved in the activation of the pituitary–adrenal axis following immune stimulation [6–8].

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EFFECTS OF INTERLEUKIN 1 ON THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

Intravenous or intracerebroventricular administration of nanogram amounts of IL1α or IL1β to rodents stimulates the pituitary–adrenal axis within 30 min [9, 10]. There is controversy at which level these cytokines stimulate this axis.

Effects of interleukin 1 on the adrenal gland

IL1 and TNF have been reported to directly modify the secretory activity of steroid-producing endocrine target organs such as testis and ovary [11, 12]. With respect to the adrenal gland very recently Natarajan *et al.* [13] reported that recombinant human TNF at a concentration of 2.5 ng/ml produced an inhibition of angiotensin II-induced aldosterone synthesis by isolated rat glomerulosa cells during incubations for 1 or 2 h. TNF also attenuated the stimulatory effect of ACTH on aldosterone synthesis *in vitro*. Recombinant human IL1β was an even more powerful inhibitor of angiotensin II-induced aldosterone synthesis, but did not inhibit ACTH-induced aldosterone synthesis. With respect to glucocorticoid secretion there is evidence that long-term incubation of adrenocortical cells with IL1 or IL6 has a stimulatory effect [14, 15]. Whitcomb *et al.* [14] reported that

recombinant human IL1 stimulated cortisol output by human adrenocortical cells by 30% after 24 h of culture, whereas Salas *et al.* [15] demonstrated that corticosterone secretion by rat adrenocortical cells increased 2-fold after incubation for 24 h by either IL1 or IL6. Roh *et al.* [16] found that acute administration of a high dose of recombinant human IL1β (3.5 μg) in the renal artery of isolated, perfused rat adrenals stimulated corticosterone secretion. Regarding TNF, Brennan *et al.* [17] reported that this cytokine did not modify basal corticosterone secretion by rat adrenocortical cells *in vitro*, but at a concentration of 1 ng/ml completely abolished ACTH-stimulated corticosterone secretion after 90 min of incubation. In a recent study [18] we showed that short-term incubation (120 min) of rat adrenocortical cells with IL1α, 1β, 2 and 6 and with TNF in concentrations up to 1 μg/ml did not significantly modify basal or ACTH-stimulated corticosterone secretion.

Effects of interleukin 1 on the anterior lobe of the pituitary gland

There is still controversy whether IL1 has a direct effect on ACTH and β-endorphin secretion by the anterior lobe of the pituitary gland. Sapolsky *et al.* [19] and Berkenbosch *et al.* [20] were unable to show a direct effect of either recombinant human IL1α or IL1β on ACTH release from cultured rat anterior pituitary cells, using concentrations up to 10^{-8} M. However, Uehara *et al.* [21] demonstrated that a concentration of 10^{-7} M recombinant human IL1β significantly stimulated ACTH release by cultured rat anterior pituitary cells. Other investigators [22, 23] found that concentrations of 10^{-9} or 10^{-10} M human IL1 stimulated ACTH release by rat anterior pituitary cells, but only after incubation periods longer than 4 h. Provocative are two papers from a single laboratory reporting that minimal concentrations (10^{-10}–10^{-12} M) of human IL1β stimulated the release of ACTH, and also of LH, TSH and GH, by isolated rat anterior pituitary cells or perifused rat pituitaries [24, 25]. It is of interest that human ACTH-producing pituitary tumors can respond to interleukin 1: Malarkey *et al.* [26] found that incubation of pituitary tumor cells of patients with Cushing's disease with 10^{-9} M IL1β for 4 h stimulated ACTH release 2-fold. Murine ACTH-producing pituitary tumors can also respond to IL1 [27–30].

Effects of interleukin 1 on the hypothalamus

As has been described above, under certain experimental conditions IL1 may exert direct effects on ACTH and β-endorphin secretion by the pituitary gland and on corticosterone secretion by the adrenal gland. However, there is increasing consensus that the main site of action of IL1 is located in the central nervous system. Interleukin 1 may stimulate the activity of CRH-secreting neurons in the hypothalamus directly or through the modulating effect of catecholamines [31] or prostaglandins [32]. Several lines of evidence now support this view:

1. Rats pretreated with an anti-CRH antiserum do not show the expected increase in ACTH release following IL1 administration [19].
2. IL1 receptors have been identified in the hypothalamus [33].
3. Intraperitoneal administration of IL1 in rats stimulates gene expression of hypothalamic CRH [34].
4. CRH concentrations in nerve terminals of the median eminence decrease in response to IL1 administration [20].
5. Concentrations of CRH in the hypothalamo–hypophyseal portal system are increased after intravenous infusion of IL1 [19].
6. IL1 stimulates the release of CRH from rat hypothalami *in vitro* [35].
7. Lower doses of IL1 are needed to stimulate ACTH secretion when given intracerebroventricularly in comparison with intravenously [32].

Very recently Katsuura *et al.* [36] provided evidence that the organum vasculosum of the lamina terminalis (OVLT), a region where the blood–brain barrier is absent, and the preoptic area are important in mediating the response of ACTH to IL1. These workers propose the following hypothesis regarding the central mechanisms of ACTH release induced by blood-borne IL1. Circulating IL1 penetrates the fenestrated endothelium of the OVLT and enters into the perivascular region, where it activates the astrocytes present in this area. These astrocytes then release PGE_2 into interstitial spaces, and PGE_2 diffuses or is transported to the preoptic area. PGE_2 activates an interneuron in the preoptic area, and the signal is transmitted to CRH neurons in the paraventricular nucleus. The activation of CRH neurons in

the paraventricular nucleus stimulates the release of CRH from nerve terminals in the median eminence into the hypophysial portal circulation, thereby stimulating the pituitary corticotrophs [36].

EFFECTS OF INTERLEUKIN 2, INTERLEUKIN 6 AND TUMOR NECROSIS FACTOR ON THE HYPOTHALAMIC–PITUITARY–ADRENAL AXIS

Recent studies indicate that administration of IL2, IL6 and TNF to laboratory animals or humans also acutely stimulates the pituitary–adrenal axis [6–8].

Denicoff *et al.* [6] demonstrated that IL2 administration in patients with cancer increased plasma ACTH levels 20-fold and plasma cortisol levels 2-fold. Interestingly, the stimulation of hormone secretion was greater in the second treatment course compared with that in the first. *In vitro* studies with IL2 did result in equivocal data. Fukata *et al.* [30] and Malarkey *et al.* [26] did not find any effect of IL2 on ACTH release by cultured murine AtT-20 cells and cultured pituitary cells of patients with Cushing's disease, respectively. In contrast, Farrar *et al.* [37] observed that IL2 stimulated the release of ACTH by AtT-20 cells, whereas Brown *et al.* [28] found that IL2 enhanced POMC gene expression in rat pituitary cells and in AtT-20 cells.

With respect to IL6, Naitoh *et al.* [7] showed that administration of a bolus injection of 5 μg IL6 in conscious freely-moving rats significantly increased plasma levels of ACTH 30 min after the injection. Immunoneutralization of CRH blocked the stimulatory effect of IL6 on ACTH secretion, suggesting that this effect of IL6 is mediated by an action on CRH-secreting neurons. In contrast, Fukata *et al.* [30] reported that IL6 stimulated ACTH release directly from cultured AtT-20 cells. Very recently it has been demonstrated that IL6 is produced by anterior pituitary cells *in vitro* [38]. There is some evidence that the source of IL6 in the pituitary gland is the folliculo-stellate cell [39]. Recently Yamaguchi *et al.* [40] demonstrated that the release of IL6 by rat anterior pituitary cells in culture is enhanced by IL1β. Together, these data suggest that IL6 plays a role as an intrapituitary releasing factor.

Sharp *et al.* [8] showed that i.v. bolus injections of TNF at doses (100–1000 ng) which failed to affect blood pressure, food consumption or prolactin levels resulted in significant elevations of plasma ACTH levels in rats within 20 min. Data concerning direct effects of TNF on ACTH secretion by anterior pituitary cells *in vitro* are conflicting: a positive effect has been claimed by Mealy *et al.* [41] and Milenkovic *et al.* [42], a negative effect by Gaillard *et al.* [43] and no effect by Sharp *et al.* [8], Bernardini *et al.* [44] and Kehrer *et al.* [23]. Also with respect to a possible central site of action of TNF the data are controversial [8, 41, 44, 45].

CONCLUDING REMARKS

After stimulation of the immune system, e.g. during infectious diseases, the activity of the pituitary–adrenal axis is increased. Research, conducted in the last few years, has provided strong, but indirect, evidence that cytokines—peptides released from macrophages and other cells of the immune system after antigenic challenge—play a role in this activation of adrenal function. The concept has emerged that cytokines (particularly IL1) and glucocorticoid hormones integrate an immunoregulatory feedback circuit: during stimulation of the immune system cytokines are released into the peripheral circulation, mediating an increase in plasma glucocorticoid levels. As a consequence, immune cell functions and production of IL1 and other cytokines will be suppressed, thereby preventing a harmful overshoot of immune reactions [46]. It has to be noted, however, that the evidence supporting the existence of such an immunohormonal regulatory feedback system is indirect. To validate this concept additional studies would be required:

- —Measurements of levels of cytokines in the blood during activation of the immune system.
- —Dose–response studies on the effects of acute administration of cytokines on the activity of the pituitary–adrenal axis, with careful attention for activation of the pituitary–adrenal axis due to aspecific stress.
- —Studies on the effects of chronic administration of cytokines in laboratory animals.
- —Studies on the effects of immunoneutralization of cytokines on the activity of the pituitary–adrenal axis during stimulation of the immune system.

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