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Tumor Necrosis Factor Alpha Differentially Regulates Beta-Endorphin Concentrations and Proopiomelanocortin RNA in the Anterior and Neurointermediate Pituitary in vivo

Key Words

Cytokines
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Abstract

We analyzed the effect of tumor necrosis factor α (TNF- α) on β -endorphin concentrations and proopiomelanocortin mRNA in the rat anterior and neurointermediate pituitaries. The intraperitoneal injection of 5 (μ g/kg TNF- α decreases β -endorphin in neurointermediate pituitocytes 4, 8 and 24 h after the treatment without affecting proopiomelanocortin (POMC) RNA. In contrast, in the anterior pituitary 4 h after the injection of the cytokine, POMC RNA was decreased while the peptide content was increased. These effects can be relevant to the modulation of the pituitary-adrenal axis and immune responses in conditions, such as infections, in which TNF levels are increased.

It has by now become apparent that important interactions exist between the immune and the endocrine systems [1]. Different cytokines have been shown to modulate pituitary hormone release by both hypothalamic and direct pituitary actions [1]. Tumor necrosis factor α (TNF), a macrophage-derived pleiotropic cytokine, is involved in the acute-phase response to inflammatory stimuli and is the main mediator of endotoxic shock and the cachexia that accompanies cancer [2]. Relevant plasmatic concentrations of TNF are reached during bacterial infections, tumor progression and other pathologies like multiple sclerosis and AIDS [2-4]. TNF can therefore be considered a signal to the brain of the state of activation of the immune system. While data exist on the modulation by TNF of prolactin [5] growth hormone [5, 6] and adreno-

corticotropin (ACTH) [5, 7, 8] secretion from pituitary cells, no information is available on the effect of this cytokine on the pituitary synthesis and release of the opioid peptide β -endorphin (BE). The opioid peptide BE is derived from a larger precursor, proopiomelanocortin (POMC) which gives rise also to ACTH, α -melanocyte-stimulating hormone and CLIP [9]. BE plays a central role in the responses to stressful situations, and it has recently been involved in immunomodulation [10]. For this reason, we analyzed the effect of peripherally injected TNF, at a concentration which can be reached in the circulation during infections or immune diseases, on BE and POMC RNA in the anterior and neurointermediate lobe of the rat pituitary.

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Materials and Methods

Male Lewis rats (180 g body weight), 9 in each experimental group, were given an intraperitoneal injection of human recombinant TNF- α (generous gift of Dr. Fields, Biogen, Gent, Belgium) at a dose of 5 ng/kg. The control group was injected with saline alone. Rats were killed 30 min, 4, 8 and 24 h after treatment.

For RNA evaluation, animals were killed by decapitation, the anterior and neurointermediate pituitary lobes were rapidly removed on dry ice, pools of 3 animals were prepared and kept at -80°C until use.

For RNA extraction, tissues were homogenized in the presence of 4 M guanidine thiocyanate, and total RNA was isolated by ultracentrifugation through 5.7 M cesium chloride as previously described [11,12].

Equal amounts of RNA samples (10 and 20 μ g) were electrophoresed through 1 % agarose gel in 1 x MOPS buffer according to standard procedures [13]. RNA was transferred to filters (ICN Biotrans-plus) and fixed by drying at room temperature. Hybridization was carried out using the cDNA probe p113 for rat POMC (gift from JX. Roberts) [14] labeled with [α -³²P]dCTP by the Megaprime DNA labeling system (Amersham).

Hybridization was performed for 48 h at 42°C, and filters were then washed in 2 x SSC/0.1 % SDS for 10 min at room temperature, followed by a wash in 0.1 x SSC/0.1 % SDS for 15 min at 55°C. Filters were then air dried, wrapped with a single layer of Saran wrap and placed in X-ray cassettes with Kodak X-Omat AR film for autoradiography. A single strong band of 1.2 kb, corresponding to POMC RNA was identified in each lane.

The hybridized radioactive probe was then removed by immersion of the nitrocellulose filter in boiling water. In order to verify the amount of total RNA loaded, the filters were hybridized again with an 18S ribosomal probe (1.9-kb *EcoRI-Sall* fragment). Densitometry analysis was performed with an LKB Ultrascan XL densitometer, and the relative intensities of the bands were estimated by weighing their densitometric profiles [15].

For BE measurements, rats were killed by microwave irradiation in order to prevent enzymatic degradation of the peptide, anterior and neurointermediate pituitaries were frozen until further processing. Pituitaries were resuspended in 1 ml of 0.1 N acetic acid, homogenized and centrifuged at 10,000 g [16]. Supernatants were collected for radioimmunoassay and pellets were utilized for protein evaluation. BE measurements were performed by radioimmunoassay according to a method previously described in detail [17]. The BE antiserum was obtained against synthetic BE(1-27) and it is directed towards the C-terminal of the peptide. It shows 100% cross-reactivity with both human and rat BE(1-31), while no cross-reactivity exists with met- and leu-enkephalin, dynorphin, α - and β -melanocyte-stimulating hormone, substance P, somatostatin, thyrotropin-releasing hormone, corticotropin-releasing hormone, morphine and naloxone. [3-(125)iodotyrosyl-27]BE was purchased from Amersham (specific activity 747 Bq/mmol). The results obtained with the use of this antibody were comparable to the ones achieved using the antiserum previously described [16].

Protein concentration was measured by the method of Lowry et al. [18].

Statistical analysis of results was performed by Anova, followed by Tukey's test for multiple comparison.

Figure 1 shows that a significant increase in BE content in the anterior pituitary is present 4 h after the injection of TNF. Eight hours after the treatment, the BE levels have already returned to basal levels. On the contrary, the cytokine significantly decreases BE concentrations in the intermediate lobe of the pituitary. The effect appears 4 h after TNF treatment, and it is still present 24 h later.

In figure 2, the effect of TNF on anterior and neurointermediate lobe POMC RNA concentrations is reported. The POMC RNA content in the anterior pituitary is slightly reduced 4 h after TNF, while the treatment with the cytokine does not modify POMC RNA levels in the neurointermediate lobe at any time considered.

Discussion

It is generally recognized that the measurement of RNA or of peptide concentration alone is not a sufficient marker of the real state of activation of a peptidergic system, while the combination of the two measurements can provide a more detailed picture of the peptide turnover. While many papers have reported on the effect of TNF on plasma ACTH levels or on ACTH release from pituitary cells in vitro [5, 7, 8], to our knowledge no data exist on the modulation by this cytokine of POMC RNA. From the data presented it appears that TNF exerts interesting and differential effects on BE in the anterior and in neurointermediate lobes. The decrease in the content of neurointermediate lobe BE induced by the cytokine is relevant and long lasting. It starts in fact 4 h after the treatment, and it is still present 24 h after the TNF injection. In contrast to the peptide, the level of RNA is never affected by TNF. It can therefore be suggested that TNF stimulates BE secretion without affecting the synthesis of the opioid. The concentrations of the peptides start in fact to slowly increase towards normal values, but 24 h after TNF the values are still significantly lower. Alternatively, TNF could affect processing and metabolism of BE within the gland, directing the posttranslational modification of the peptides towards molecular forms such as 1-17 BE (a-endorphin) or 1-16 BE (y-endorphin) which cannot be detected by our antibodies, since the C-terminal of the peptide is lacking.

More difficult to explain are the effects exerted by TNF on the anterior pituitary, since we observed an increase in the peptide coupled to a decreased level of RNA. The effect is however weak and transitory, in comparison to the effects on the neurointermediate lobe. It is

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Fig. 1. Effect of TNF-a treatment on BE concentrations in anterior pituitary (a) and neurointermediate pituitary (b). Control animals were injected with saline. Values represent the mean ± SD of 8 animals, *p < 0.01, compared with control animals.

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Fig. 2. Graphic analysis of anterior (a) and neurointermediate (b) pituitary POMC RNA; 10 and 20 ug of total RNA were analyzed with both a POMC cDNA probe and an 18S probe. Autoradiographs were scanned using an LKB ultrascan laser densitometer and analyzed using LKB Gelscan XL software. The graph represents means $\pm$ SD of 2 separate experiments, each of which consisted of analysis of 3 pools of tissue (3 rats/pool).

in fact present only 4 h after the treatment. This pattern can be justified only on the basis of a sudden inhibition exerted by TNF on both release and synthesis of BE.

It is interesting to note the differential effects exerted by TNF on ACTH and BE in the anterior pituitary: while a stimulation by TNF of ACTH release has been described, we show an inhibitory modulation of BE and POMC. The widely described increase in ACTH plasma concentrations induced by TNF [5,7, 8] seems therefore to be due only to the stimulation of ACTH secretion, since the POMC precursor, which gives origin

to both ACTH and BE, according to our results, is only slightly affected by the treatment. TNF can therefore be considered only a secretive stimulus for the POMC products, i.e. BE from the neurointermediate lobe and ACTH from the anterior pituitary. It remains to be established, however, whether TNF exerts its modulatory activity either acting directly on pituitary POMC or throughout the mediation of the many releasing factors and neurotransmitters which participate in the regulation of pituitary POMC. The involvement of corticotropin-releasing hormone in the effects of TNF can be excluded since it is a very potent stimulator of both release and synthesis of BE [9] in the anterior pituitary. The noninvolvement of corticotropin-releasing hormone in the TNF effect on POMC rules out also a possible involvement of interleukin-1, a

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cytokine which is stimulated by TNF [2, 19]. Interleukin-1 is in fact a potent activator of the hypothalamic-pituitary-adrenal axis, but its effect is mediated by hypothalamic corticotropin-releasing hormone [1]. Also the mediation of the dopaminergic, adrenergic and GABAergic systems can be ruled out, since their effects on the pituitary POMC do not coincide with those of TNF [9].

The inhibitory effect exerted by TNF on anterior pituitary POMC and BE resembles that of glucocorticoids [20]. TNF has been shown to stimulate glucocorticoid secretion both by a direct effect on the adrenal gland [2] and by ACTH secretion. It can therefore be suggested that TNF induces a fast release of glucocorticoid, and thereafter these hormones could exert an inhibitory modulation on anterior pituitary POMC. Further experiments are needed in order to better elucidate this point.

The effect of TNF on POMC and BE can be relevant in pathological conditions such as infections or tumor progression [2], during which TNF plasma levels reach high concentrations.

Interestingly, modifications of TNF production have also been reported in pathologies involving mainly the neuroendocrine system, i.e. anorexia nervosa [21], in which the p-endorphinergic system is deeply involved [22].

Recently, it has been found that TNF is produced also in the brain by microglia and astrocytes upon stimulation by viruses or interferon- γ [23], or during autoimmune disease such as multiple sclerosis [3]. As a consequence, in these pathological conditions not only circulating TNF but also glia-derived TNF could influence pituitary hormones.

The relation TNF-BE could therefore be active in many different situations and pathologies in which the neuroendocrine and the immune system are strictly interconnected.

Finally, it can be speculated that by affecting the hypothalamic-pituitary-adrenal axis, TNF can end up in modulating the immune function, also modifying corticosteroid-al secretion and affecting the release of BE, which has been shown to participate in the immune regulation [10,24].

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