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Endothelial immunocytochemical expression of pituitary IL-1β and its relation to ACTH-positive cells is regulated by corticosterone in the male rat

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ABSTRACT

Interleukin-1 beta (IL-1β) is a cytokine linking the neuroendocrine system and metabolic homeostasis. We have previously demonstrated the relevance of IL-1β for maintaining the pituitary ACTH-producing cells by immunoblocking its effects in pituitary cultures. However, the morphological characteristics and the intimate relationship of the pituitary cells expressing IL-1β and ACTH remain unknown. For determining pituitary variations of immunoreactivity for IL-1β and its relation with ACTH-positive cells under stress situations, we performed an immunohistochemical analysis of the expression of IL-1β and ACTH in the pituitary gland of adult rats, in the absence or presence of corticosterone, by establishing different groups: untreated, sham-operated, and bilaterally adrenalectomized animals. In the rats subjected to surgery, the glucocorticoid was administered on the same day of the intervention and on the third day post-surgery. Interestingly, it was observed that IL-1β was located in the pituitary endothelial cells at the hypophyseal portal vessels, regardless of the treatment schedule. When comparing the pituitary immunoreactive surface to IL-1β expression without corticosterone, adrenalectomized animals displayed a significantly greater area than the sham-operated animals. Corticosterone significantly inhibited the effect of adrenalectomy depending on the time interval it was administered. By in situ hybridization, IL-1β mRNA expression was also correlated with immnunocytochemical expression of pituitary IL-1β. Our results demonstrate that IL-1β is a constitutive element in endothelial portal pituitary vessels and under stress experimental conditions IL-1β increases its expression and its relation with ACTH-positive cells, suggesting that IL-1β could participate in an autocrine-paracrine fashion thereby modulating the pituitary population of ACTH-positive cells.

1. Introduction

The interleukin-1 family of cytokines encompasses several polypeptides involved in systemic pathways related to the immune and endocrine system, and also participates in cell growth, differentiation and function [1]. Furthermore, the relevance of IL-1β, a cytokine related to the inflammasome, which links the regulation of inflammatory responses and immunomodulation with the neuroendocrine system and metabolic homeostasis, is noteworthy [2–4].

IL-1β is released by different cellular types and, as a proinflammatory cytokine, many of its relevant actions are implicated in the modulation of the HPA axis [5–8].

The pituitary synthesizes both IL-1β and its receptor, as their mRNA and protein expression have been demonstrated [9–12].

Because IL-1β and its specific receptor are present in the pituitary gland [13] autocrine-paracrine effects have been attributed to this

Abbreviations: ACTH, adrenocorticotropic hormone; ADX, adrenalectomized animals without corticosterone; CVO, circumventricular organs; DAB, 3,3′-diaminobenzidine; ECs, endothelial cells; HPA, hypothalamus-pituitary-adrenal; HRP, horseradish peroxidase; ICE, interleukin-1 beta converting enzyme; IL-1β, interleukin-1 beta; IL-1R1, interleukin-1 receptor type 1; LPS, lipopolysaccharide; PAP, peroxidase-antiperoxidase; PBS, phosphate buffered saline; POMC, proopiomelanocortin; TB, trizma-HCl buffer; TBS, trizma-HCl buffered saline

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hypophyseal IL-1β, such as a regulator of hormonal secretion, together with the differentiation, proliferation and apoptosis of several pituitary cells [14–18].

Concerning the regulation of the HPA axis, IL-1β stimulates the secretion of POMC derivatives thus promoting the release of ACTH [16,19–21]. Indeed, the relationship between the hypophyseal IL-1β and the HPA axis follows a bidirectional feedback pattern [5,6,22], including the notorious influence regarding the time course, and correlation with an autocrine-paracrine regulation at the pituitary level [12,23]; although, some aspects concerning the cellular characteristics or local variations still remain unknown.

The question of how this network implicates both the pituitary IL-1β and the HPA axis, considering their bidirectional autocrine-paracrine regulation and their participation in the maintenance of populations of relevant cellular elements involved in these processes, has been of particular interest in our own research [17,18,24].

Therefore, in order to answer questions regarding this and to complement our previous findings, the aim of this study was to elucidate the characteristics and variations of the pituitary cells that might show immunoreaction to IL-1β, by analyzing groups of control, simulated or adrenalectomized adult rats. Additionally, a possible correlation with the pituitary immunocytochemical expression of ACTH was also studied in these animals. Following a brief period after the administration of corticosterone to the groups of rats submitted to their corresponding surgical procedure, and by using double immunohistochemical labeling for ACTH and IL-1β respectively, possible modifications in both the pituitary ACTH-positive cells and IL-1β-positive cells were analyzed.

2. Materials and methods

2.1. Animals and experimental protocol

The experiments were carried out following the protocols and ethical requirements approved by the Committee for the Care and Use of Animals of the University of Salamanca, in accordance with the regulations for the use of animals in investigation procedures from the European Communities Council Directive (2010/63/EU) and the current Spanish legislation (RD 53/2013).

For all the experimental procedures, adult male Sprague-Dawley rats 8 weeks-old and weighing 175–200 g were used. The animals were individually housed under controlled conditions, in a room maintained at 21 ± 2 °C and 50 ± 5% of relative humidity, with a controlled cycle of 14 h light – 10 h darkness. A balanced standard rat diet (Panlab®, Barcelona, Spain) and water were freely available. The groups of animals used for the experiments were as follows (Table 1):

Untreated rats. 5 phenotypically normal rats without any treatment.

Sham-operated (simulated). 40 rats of this group were submitted to surgery although, after laparotomy, the adrenal glands were localized but not removed, and no other surgical procedure was carried out. The animals were sacrificed after 2, 4 or 6 days of surgery, 5 rats per time interval, and the other 25 rats were treated with corticosterone as later described.

Adrenalectomized rats. 40 rats were bilaterally adrenalectomized by a dorsal extraperitoneal laparotomy under ketamine anesthesia (10 mg/kg body weight, administered intraperitoneally); after the surgical procedure, the drinking water was supplemented with 0.9% NaCl and 5% sucrose, as previously described [25]. The animals were sacrificed 2, 4 or 6 days after surgery, 5 rats per time interval, and the other 25 rats were treated with corticosterone as later described.

Rats treated with corticosterone. 50 rats, 25 sham-operated and 25 adrenalectomized, were treated with corticosterone (1.5 mg/kg body weight b.i.d, intramuscularly/12 h). In every surgical circumstance two groups were established. One group was treated starting from the same day of the surgery (day 1) until the day of sacrifice, which occurred on days 2, 4 or 6. In the second group, the treatment with corticosterone started on the 3rd day after surgery (day 3) and animals were sacrificed on days 4 or 6. For every time point, 5 rats were studied.

Table 1
Scheme of the experimental protocol.

Groups of animals	Corticosterone treatment 1.5 mg/kg body weight twice daily (number of rats used is indicated)			Survival time (days after surgery)
	No	Yes[a]		
		Day 1	Day 3	
Untreated	5	–	–	Not applied
Sham-operated	5	–	–	2
	5	–	–	4
	5	–	–	6
Adrenalectomy	5	–	–	2
	5	–	–	4
	5	–	–	6
Sham-operated	5	5	–	2
	10	5	5	4
	10	5	5	6
Adrenalectomy	5	5	–	2
	10	5	5	4
	10	5	5	6

[a] Following the surgical procedure, the glucocorticoid was administered on the same day of surgery (Day 1) and on the third day after the intervention (Day 3) for the indicated groups.

2.2. Sample processing

After the corresponding treatment period, the animals were anesthetized with isoflurane inhalation and were sacrificed by decapitation between 10 am and 11 am. Immediately, the pituitary glands were carefully dissected and fixed in a solution of paraformaldehyde 4% in phosphate buffer (0.1 M, pH 7.4) for 24 h. Then, the samples were dehydrated in ethanol, cleared in xylene, and embedded in paraffin for making serial coronal sections with a thickness of 5 μm.

2.3. Immunohistochemistry

For single immunostaining, the streptavidin-peroxidase method was used; in double immunostaining, the streptavidin-peroxidase method was used followed by the peroxidase-antiperoxidase (PAP) reaction. The slides were previously deparaffinized and rehydrated. The endogenous peroxidase was blocked by incubating with 0.25% H_2O_2 in methanol for 30 min. Afterwards, they were washed three times in TBS (Trizma-HCl buffered saline 0.05 M, pH 7.4, plus 0.9% NaCl, used as the solution for washes and dilutions). The non-specific reaction of the secondary antibody was blocked by incubation in normal goat serum (Dako®, Glostrup, Denmark, diluted 1:30 in TBS) for 30 min.

The single immunostaining method was performed to detect IL-1β expressing cells: the sections were incubated in the primary antibody, polyclonal rabbit anti-rat IL-1β (Endogen®, Endogen Inc., Woburn, MA, USA, diluted 1:120 in TBS) for 24 h at 4 °C in a humidity chamber. After washing, the slides were incubated for 45 min at room temperature with a biotinylated-goat anti-rabbit IgG (Caltag®, San Francisco, CA, USA, diluted at 1:150 in TBS) and then for 30 min at room temperature with streptavidin-horseradish peroxidase complex (Caltag®, diluted at 1:250). Then, the sections were washed again with TBS, and the reaction was revealed using a freshly prepared solution of 3,3′-diaminobenzidine tetrahydrochloride (DAB; Sigma®, Sigma-Aldrich Inc., St. Louis, MO, USA), at 0.025% in TB (Trizma-HCl buffer 0.05 M, pH 7.4), plus 0.03% H_2O_2. The samples were counterstained with Mayer's hematoxylin.

In order to confirm the specificity of the immune reaction, substitution of the primary antibody by TBS or non-immune rabbit serum,