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Prenatal expression of interleukin 1β and interleukin 6 in the rat pituitary gland

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ABSTRACT

It is known that interleukin 1β (IL-1β) and interleukin 6 (IL-6) are expressed post-natally in normal and tumoral cells in the anterior pituitary, and that they play a role in both the liberation of different hormones and in the growth, proliferation and tumor formation of the pituitary gland. However, their expression and role during embryonic and fetal development remain unknown. We have performed an immunocytochemistry study of prenatal expression and distribution of IL-1β and IL-6 in isolated embryonic rat Rathke's pouch prior to birth, more specifically between 13.5 and 19.5 days p.c. Western-blot analysis carried out on 19.5-day p.c. embryos showed positive immunolabelling for IL-1β and IL-6. These interleukins were initially expressed simultaneously in the rostral and ventral portions of Rathke's pouch in 15.5-day p.c. embryos, and this expression progressed caudodorsally in later developmental stages, extending to most of the hypophysis before birth. The number of cells expressing these interleukins increased throughout this period: 48.22% of anterior pituitary cells expressed IL-6 in 19.5-day embryos, whilst IL-1β was positive in 39.8% of the cells. Moreover, we have demonstrated that some adenohypophyseal cells co-express both interleukins. Such findings represent the first step towards an understanding of the physiological role of these interleukins in anterior pituitary development.

1. Introduction

1.1. Development of the adenohypophysis in rat

Adenohypophysis (anterior, intermediate and tuberal lobes of the pituitary gland) develops from the Rathke's pouch as an invagination of the epithelium in the roof of the stomatodaeum (primitive buccal cavity), which will isolate from it at 13–13.5 days post-coitum (p.c.). The Rathke's pouch will contact with the floor of the diencephalon, inducing the formation of the neurohypophysis (pars nervosa) and the infundibulum (pituitary stalk). The ventral wall of the Rathke's pouch undergoes intense growth, forming the pars anterior (anterior lobe) of the adenohypophysis; subsequently, dorsal and lateral prolongations or wings develop on each side, which reach the pituitary stalk and will form the pars tuberalis (tuberal lobe); following this, the dorsal wall of the Rathke's pouch undergoes small-scale cellular proliferation, with the formation of the pars intermedia (intermediate lobe) of the adenohypophysis.

1.2. Interleukin expression and function in normal and tumoral adult pituitary cells

Interleukins are multifunctional peptides produced by many different types of cells and tissues, including principally the immune and endocrine systems. Numerous studies, employing immunocytochemistry, mRNA detection and "in situ" hybridization, have shown that interleukin 1β (IL-1β), interleukin 6 (IL-6) and their receptors are expressed in normal adenohypophysis cells in different species of animals. It has been demonstrated that IL-1β is diffusely expressed in pituitary cells of the anterior lobe of the hypophysis, especially in TSH-secreting cells [1–3], and that IL-6 is localized in ACTH, gonadotropin, GH and PRL-secreting cells, as well as in folliculo-stellate cells (non-secreting cells) [4,5]. In addition, the IL-1 receptor type1 (IL-1R1) has been detected in cells from the anterior lobe of the pituitary gland [6,7], mainly in GH-secreting cells and, to a lesser extent, in PRL and ACTH-secreting cells [8]. The receptors for IL-6 have also been detected in normal pituitary cells, specifically in GH, FSH–LH, PRL and ACTH-secreting cells [4,9,10].

Cytokines could also be involved in pituitary cellular proliferation and growth [11]. It seems that IL-1β and IL-6 inhibit the growth of normal rat pituitary cells, but both cytokines and their receptors have been shown to exist in different cell lines in pituitary adenomas [4,9,12,13], which could be indicative of their

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involvement in pituitary gland tumor formation [14–16]. Although IL-1β does not seem to be directly related to pituitary tumorogenesis [13], it has been demonstrated that IL-6 is involved in the development of diverse pituitary adenomas [14,15,17–19].

Regarding the physiological role of cytokines in the adenohypophysis, it is believed that some interleukins may have a role to play in the release of certain pituitary hormones [2,20]; in this regard, we know that peripheral administration of IL-1β, IL-2 and IL-6 increases plasma levels of ACTH [1,21,22], whilst central administration of IL-1β produces intense inhibition of pituitary secretion of LH and FSH [23,24]; similarly, IL-6 stimulates PRL, GH, FSH and LH liberation in cell cultures of rat pituitary cells [25,26] and might contribute to excessive GH production in the majority of somatotroph adenomas [27].

1.3. Interleukin expression and function during embryonic development

Despite both our knowledge of cytokine expression in the anterior pituitary and, as previously mentioned, the abundant data relating to its functional role, most studies concern the post-natal period, with little information relating to the possible role of interleukins in adenohypophyseal development during the embryonic and fetal periods. Nowadays it is known that several interleukins are expressed in normal embryonic cells at very early stages of development [28–30]; we have previously demonstrated that IL-1β is expressed in the neuroepithelium of the embryonic spinal cord and stimulates proliferation and differentiation in these cells [31]. Moreover, receptors for IL-6 and IL-8 have been detected in numerous tissues and organs in human foetuses [32]. The expression of receptors for the Leukemia Inhibitor Factor (LIF), IL-6 and of the subunit gp130 in human fetal pituitary tissue between 18 and 31 weeks of gestation has been demonstrated [33], and it has also been shown that LIF and IL-6 stimulate ACTH and GH secretion in primary cultures of human fetal adenohypophyseal cells. Furthermore, when the subunit gp130 was blocked by antibodies, the levels of ACTH dropped below basal values, indicating that LIF and IL-6 may be involved in regulating pituitary function during the fetal period via the same transduction signal. In addition, it has been proven that lipopolysaccharides from gram-negative bacteria induce permanent neuroendocrine changes by the liberation of both cytokines IL-1β and IL-6 during the prenatal [34,35] and neonatal periods [36]. All of this suggests that cytokines could play a physiological role in the normal development of the anterior pituitary, and that variations in levels of interleukins during embryogenesis might disturb neuroendocrine regulating programmes, with subsequent consequences in adult life.

Given the scant knowledge concerning the possible influence of cytokines on normal pituitary development, in this paper we have employed immunocytochemistry and western-blot to investigate the chronological and topographical pattern of IL-1β and IL-6 expression in isolated Rathke's pouch of rat embryos until immediately prior to birth. This represents an initial step towards understanding the physiological function these interleukins may have in anterior pituitary development.

2. Materials and methods

2.1. Obtaining rat embryos

Wistar rats, stabulated at a constant temperature (22 °C) and with a cycle of 12 h of light and 12 h of darkness, were employed. To calculate embryonic age, day 0 referred to the morning when a copulation plug was found after overnight mating. Pregnant rats were sacrificed by ether inhalation and the embryos extracted from the uterus; the enveloping membranes were then removed and their heads processed for histology. For our study, embryos of between 13.5 days p.c.—immediately after the isolation of Rathke's pouch from the roof of the stomatodaeum—and 19.5 days p.c. were employed. Five embryos from each stage were used for immunocytochemistry (35 embryos in total).

2.2. Immunocytochemistry

The heads of the embryos were fixed in picric acid–paraformaldehyde [37] for 12 h at 4 °C. Following this, the specimens were dehydrated through a graded series of ethanol and embedded in paraffin through xylene. Subsequently, we made 8 μm-thick serial coronal sections, which were mounted on glass slides. These were then deparaffinized, rehydrated and submerged consecutively in lugol and sodium metabisulphite in order to eliminate the fixing agent, and afterwards washed several times in PBS and the endogenous peroxidase inhibited with a solution of methanol and 3% hydrogen peroxide for 30 min. After several washings in PBS, the sections were incubated in PBS/BSA for 20 min and subsequently in the first antibody, IL-1β rabbit anti-rat polyclonal or IL-6 rabbit anti-rat polyclonal (Endogen). In both cases, the conditions were the same, namely, a 1/100 dilution in PBS at 4 °C (a working dilution of 10 μg/ml) overnight in a humid chamber. Following washing in PBS, we proceeded to incubate with the secondary antibody—horse radish peroxidase-conjugated goat anti-rabbit IgG (Southern Biotechnology Associates, Inc.)—at a dilution of 1/100 in PBS, at room temperature for 90 min. After further washing in PBS, the sections were incubated with 3.3́-diaminobenzidine tetrahydrochloride (DAB; Sigma) and H_2O_2 (DAB 10 mg/15 ml in 0.05 M Tris–HCl buffer, pH 7.4, and 0.003% H_20_2). Haematoxylin was employed to counterstain the nuclei. The control sections were prepared as mentioned above in order to prove the specificity of the immunoreaction, but now using pre-immune serum instead of the primary antibody. The tissue sections were observed with a Nikon Microphot-FXA Photomicroscope and the most representative were photographed.

In order to verify whether the adenohypophyseal cells simultaneously express IL-1β and IL-6, we carried out a double immunolabelling on sections of 18.5 days p.c. rat embryo adenohypophysis. Fixation, dehydration, embedding and section of embryos were similar to previously described. The sections were deparaffinized, rehydrated and washed several times in PBS, and then they were incubated in a solution of primary antibodies, Rabbit anti-Rat IL-1β polyclonal (Chemicon) and Goat anti-Rat IL-6 polyclonal (R&D Systems), both at a final concentration of 15 μg/ml in PBS, at 4 °C overnight in a humid chamber. After washing several times in PBS, sections were incubated with a mixture of the secondary antibodies, Goat anti-Rabbit IgG conjugated with fluorescein isothiocyanate (FITC) (Chemicon) for IL-1β, diluted 1/64 in PBS, and Donkey anti-Goat IgG conjugated with Alexa-594 (Molecular Probes) for IL-6, diluted 1/1000 in PBS. After that, sections were washed in PBS and mounted in Aquamount (Gurr). They were observed with a confocal microscope Zeiss LSM-310, and the most representative samples were photographed. Control sections were prepared as described above, but using non-immunized animal IgG instead of one of the primary antibodies (Rabbit IgG for IL-1β and Goat IgG for IL-6), and there was no cross-reaction between antibodies.

2.3. Counting and locating the immunostained cells

The immunostained cells for each interleukin were counted (100×) from a total of 4000 ± 279.29 cells; there were 50 randomly-selected fields from the same region, with 80 ± 5.59 cells per field, along the whole of Rathke's pouch. Four different embryos were used for each stage studied and the data were ex-