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Morphophysiology of the Zuckerkandl's paraganglion: Effects of dexamethasone and aging

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Abstract

The extra-adrenal Zuckerkandl's paraganglion is used as a source of chromaffin cells for transplantation in parkinsonian animals. Aging can affect its viability, and this tissue needs further characterization for improving grafting procedures. The objectives were: (i) to compare the main morpho-functional characteristics of prepubertal and old Zuckerkandl's paraganglion (ZP), and (ii) to discern phenotypic changes after sub-chronic dexamethasone treatment in extra-adrenal tissue of prepubertal rats. For these purposes, immunostaining methods, stereology, voltammetry, cell culture, Western blotting, and ELISA were employed. The findings revealed that all paraganglia were composed of mesenchymal tissue and chromaffin cells. In prepubertal rats, chromaffin cells are arranged as large or small clusters. Large clusters (also known as "cell nests") contain densely packed chromaffin cells, and they are seen as fascicles in longitudinal sections. In old paraganglia, cell nests disappear, and chromaffin cells are found to be arranged as small cell clusters or dispersed throughout the mesenchyma. Paraganglionic chromaffin cells possess a rounded morphology with diameter ranging from 12 to 15 μm, with intracytoplasmic granules (100–500 nm in diamater) containing catecholamines. Prepubertal and old ZP chromaffin cells are mostly noradrenergics, and a few of them are dopaminergics. Aging reduces the amount of chromaffin tissue (28% in adult rats vs. 11% in old animals, both in relation to total volume of the paraganglion), and induces the presence of adrenergic cells and adrenaline. Both prepubertal and old cells express the neurotrophic factors GDNF and TGF-β_1, aging leading to reduced levels of both growth factors. Dexamethasone (50 μg/kg daily, 5 days) leads to the expression of phenylethanolamine-N-methyl-transferase in prepubertal paraganglia, and to a higher content and release of adrenaline.

Keywords: Chromaffin; Paraganglion; Zuckerkandl; Extra-adrenal; Noradrenaline; Dopamine; Adrenaline; GDNF; TGF-β_1

1. Introduction

Extra-adrenal chromaffin cells belong to the sympatoadrenal (SA) cell lineage, and they are located in the so-called paraganglia. Extra-adrenal paraganglia are found adjacent to the adrenal gland, on the abdominal sympathetic region (solar plexus), next to the genitals glands, and on the low abdominal aorta (the Zuckerkandl's paraganglion or ZP). Paraganglia are known to be mostly made up of mesenchyma and chromaffin cells forming small or large clusters. Large clusters (also known as "cell nests") contain densely packed chromaffin cells, and they are seen as fascicles in longitudinal sections. This arrangement of chromaffin cells was originally described by Kohn in 1903, who named fascicles and nests as "Zellsträngen" and "Zellballen", respectively (Kohn, 1903; Tischler, 1990, 2002). Paraganglionic chromaffin cells possess a rounded morphology and a diameter ranging from 12 to 15 μm, with many intracytoplasmic granules containing catecholamines. As SA paraganglionic cells located outside of the adrenal gland, noradrenaline (NA) is their main neurotransmitter, representing the 90% of the total catecholamine content, and most chromaffin cells express dopamine-beta-hydroxylase (DBH), the noradrenaline synthesizing enzyme (Coupland, 1980; Bohn et al.,

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1982). Paraganglia chromaffin cells also possess several peptides, chromogranins and trophic factors, which constitute the so-called "cocktail" secretion of paraganglia (Unsicker et al., 1996; Tischler, 1990; Unsicker, 1993; Unsicker and Krieglstein, 1996). Among these molecules, it can be found chromogranins A and B, neural cell adhesion molecules (NCAMs), growth associated protein 43 (GAP-43), adhesion molecule L1 (L1), vesicular monoamine transporter-2 (VMAT-2), glial cell-derived neurotrophic factor (GDNF), transforming growth factors β (TGFβs), and interleukines.

The paraganglia are a second source (the main one is the adrenal medulla) of circulant catecholamines, which are released into blood in response to chemical stimuli (mainly on stressful situations). Unlike adrenal medulla, paraganglionic chromaffin cells are poorly innervated (Hervonen, 1971), and they mostly respond to chemical rather than to synaptic signals (Hervonen, 1971; Tischler, 1990, 2002; Fernandez-Espejo et al., 2003). Among extra-adrenal paraganglia, the Zuckerkandl's paraganglion is the biggest one, and it was described by Zuckerkandl (1901). In mammals, ZP is located between the emergence of the inferior mesenteric and iliac arteries (Zuckerkandl, 1901; Testut and Latarjet, 1978; Ahonen et al., 1987), this organ being critical for the normal development of the cardiovascular system (Tian et al., 1997, 1998). In humans, there are two ZPs (10–20 mm in length), even though the presence of small accessories ones has also been reported (Testut and Latarjet, 1978; McNicol, 2006). There is usually one ZP in rats (3–6 mm in length), although two or more accessories smaller paraganglia can also be found (Fernandez-Espejo et al., 2001). As typical chromaffin cells, ZP cells react with potassium dichromate (classical Orth's reaction), and contain chromogranins. ZP chromaffin cells also express tyrosine-hydroxylase (TH), the dopamine synthesizing enzyme, and dopamine-beta-hydroxylase (DBH), but lack phenylethanolamine-N-methyl-transferase (PNMT), the adrenaline synthesizing enzyme (Tischler, 1990; Bohn et al., 1982; Teitelman et al., 1984). However, the presence of adrenaline has been detected by indirect methods in newborn and aged rats (Piñón et al., 1999; Villanueva et al., 2003), suggesting that PNMT could be expressed by ZP at some ages, a fact that deserves further study.

The ZP is being currently used in our laboratory as a source of chromaffin cells for transplantation in animal models of Parkinson's disease. Extra-adrenal chromaffin cells show a better survival rate than adrenal chromaffin cells after intrabrain grafting, and this long-term cell survival allows a sustained neurorestorative action (Fernandez-Espejo et al., 2001; Galan-Rodriguez et al., 2008). This neuroregenerative action is due to the trophic and regenerative action of GDNF and TGF-β_1, two dopaminotrophic factors expressed and released by ZP chromaffin cells. Dopamine release seems not to affect the grafting outcome because dopamine content and release is very low in ZP chromaffin cells (Galan-Rodriguez et al., 2008). GDNF is known to protect dopaminergic neurons from degeneration in vitro and animal models of PD, when delivered by intraventricular injections or via transplanted cells or viruses (Lin et al., 1993; Tomac et al., 1995; Beck et al., 1995; Tseng et al., 1997; Hagg, 1998; Krieglstein et al., 1998a; Gash et al., 1998; Kirik et al., 2000; Ericson et al., 2005; Villadiego et al., 2005; Wider et al., 2006), and it induces sprouting of dopamine axons (Batchelor et al., 2000). TGF-β_1 is also known to protect dopaminergic neurons when delivered in vitro (Unsicker et al., 1996), and also acts as a cofactor which potentiates the neurotrophic actions of GDNF in vitro and in vivo (Krieglstein et al., 1998b; Schober et al., 1999).

The work on this type of transplant is preliminary and further preclinical studies are needed before testing its clinical applicability. Although the morphology and functionality of prepubertal and young chromaffin cells has been studied in our laboratory, it is important to elucidate their functional response to chemical stimuli such as stress hormones, as well as to know the morpho-functional characteristics of old extra-adrenal chromaffin cells. The reason for this is that ZP auto-grafting is expected to be carried out in the elderly population (that mostly suffering from PD), and it is crucial to discern the presence and amount of dopaminotrophic factors in old ZPs, along with their catecholamine content. For all these reasons, the objectives of the present study were: (i) to compare the main morpho-functional characteristics of prepubertal and old Zuckerkandl's paraganglion (ZP), and (ii) to discern phenotypic changes after sub-chronic dexamethasone treatment (pharmacological stress) in extra-adrenal tissue of prepubertal rats. For these purposes, immunostaining methods, stereology, voltammetry, cell culture, Western blotting, and ELISA were employed.

2. Methods

2.1. Animals and protocol

Male Wistar rats (prepubertal, 30–45-day-old rats, 150–200 g; old, 18–20-month-old-rats, 900–1000 g) from the breeding colony of the Faculty of Medicine of the University of Seville, Spain, were used. Laboratory temperature was kept at 22 ± 1 °C, and a 12-h light-dark cycle (lights on at 08:00 h) was maintained throughout the experiment. Food (lab chow) and water were available ad libitum. The experimental design is shown in Fig. 1. ZPs were obtained from prepubertal and old Wistar rats, and prepubertal rats could be naïve or subjected to sub-chronic dexamethasone treatment (daily vehicle or 50 μg/kg dexamethasone, 5 days). Dexamethasone was purchased from Tocris Biosciences (UK), and dissolved in 10% DMSO/90% double distilled water.

2.2. Extirpation of the Zuckerkandl's paraganglion

Extra-adrenal paraganglia were obtained from prepubertal and old rats under anaesthesia (ketamine, 50 mg/kg IM, and xylazine, 10 mg/kg IM, Sigma). Abdominal skin