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Upregulation of vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) expression in stellate ganglia of children with congenital cardiovascular lesions

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Summary. The distribution patterns of vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide (CGRP) immunoreactivities (IR) in stellate ganglia of human neonates and infants with congenital heart and vascular lesions were investigated by the method of indirect immunohistochemistry. The results demonstrated upregulation of VIP and CGRP expression in principal ganglionic neurons independently of the type of lesion. It is suggested that the activation of neuropeptide synthesis in stellate ganglia is a compensatory reaction of ganglionic neurons in response to congenital cardiovascular lesions, in regulation of heart contractility, and as a trophic influence on the ischemic myocardium. Hypoxia is the main inducing factor for the upregulation of VIP and CGRP expression in sympathetic neurons.

Key words: Children – Sympathetic ganglia – CGRP – VIP – Congenital cardiovascular lesions

Introduction

The neuropeptides possess many biological effects (Benaroch 1994) and play an important role in autonomic regulation of ontogeny and disease (Burnstock 1990). Moreover, during the development of the mammalian autonomic nervous system not only the intensity of neuropeptide expression, but also their functional roles in nerve and somatic cells may change (Gordon et al. 1993). The vasoactive intestinal polypeptide (VIP) and calcitonin gene-related peptide belong to the group of regulatory peptides and are involved in the regulation of cardiac functions and coronary blood flow (Wharton and Gulbenkian 1987) as well as affect trophic and inductor processes in developing cells (Pincus et al. 1994; Garcia-Hirschfeld et al. 1994; Salo et al. 1995; Klimaschewski 1997). These peptides are found in human sympathetic ganglionic neurons (Baffi et al. 1992) and in the heart (Wharton et al. 1990). The results of our previous investigations indicated that relative fetal hypoxia may be the main inducing factor for neuropeptide synthesis activation. Therefore, we decided to determine whether the upregulation of vasoactive intestinal polypeptide and calcitonin gene-related peptide expression in stellate ganglionic neurons of children with hypoxic conditions are due to the hypoxia secondary to congenital cardiovascular lesions.

Material and methods

Autopsy samples of stellate ganglia obtained from 2 neonates at 5 and 8 postnatal days with hypoplastic left ventricles and Tetralogy of Fallot, respectively and 2 children at 4 years with aortal stenosis were examined. For control purposes autopsy material of stellate ganglia of 3 neonates and 3 children of similar age was used. All control specimens were free of genetic and congenital malformations. The postmortem delay varied from 1 to 8 hours. The ganglia were fixed in Zamboni's fixative solution for 1–5 days at 4 °C. Subsequently, tissue samples were rinsed in 0.1 M sodium phosphate buffer at pH 7.4 (PBS), 50% ethanol, PBS and stored in 20% sucrose at 4 °C overnight. Cryostat sections of 10 μm were mounted on chrome alum-gelatine coated slides. The

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sections were air-dried for 30–60 min, washed in PBS and treated with 10% normal goat serum (Dakopatts, X907) for 30 min in a dark humid chamber. The normal goat serum was drained and primary antibodies to VIP (Affinity, VA 1285, 1:400) or CGRP (Peninsula, IHC 6012, 1:200) were applied for 24 h. Subsequently, the sections were rinsed with PBS and covered with Cy 3™ conjugated goat anti-rabbit IgG (Jackson, 111-165-005, diluted 1:100) for 2 h. The sections were rinsed with PBS and covered with glycerin/PBS. The ganglia were analyzed using a fluorescence photomicroscope (Zeiss, Axiophot).

Results

Immunofluorescent analysis showed a considerable population of VIP and CGRP positive neurons in stellate ganglia of all children with congenital cardiovascular lesions. The VIP-IR neurons in neonates and infants comprised almost all (up to 95%) of the neuronal population of the ganglia (Fig. 1 a, c). These neurons had a different size of perikarya and were located throughout the ganglia. Some of them demonstrated short processes (Fig. 1 a). Others appeared to have small spinous protrusions extending from the soma (Fig. 1 c). Delicate VIP positive nerves with varicosities and perineural nets were revealed around neurons too (Fig. 1 a, c). However, there were differences in intensity of neuronal fluorescence between the ganglia of neonates and infants – the latter showed stronger and more uniform VIP immunoreactivity (Fig. 1 a, c).

The similarity in distribution patterns to CGRP-IR were marked in stellate ganglia of neonates and infants with congenital heart defects, but the number of CGRP positive nerve cells was relatively less – up to two-thirds of the neuronal population. These CGRP-IR neurons differed in sizes of cell bodies and were found solitarily or in clusters of up to 3–5 cells (Fig. 2 a, b). Sometimes CGRP immunopositive neurons demonstrated delicate processes or small spinous protrusions extending from their soma (Fig. 2 a, b). The CGRP immunoreactive nerve fibres were also distributed in different areas of the ganglia (Fig. 2 a, b). In control samples of stellate ganglia, both VIP and CGRP immunopositive nerve cells comprised up to one-fifth of the total neuronal population in neonates and less than one-tenth in infants (Fig. 1 b, d; 2 b, d). This data agreed with our previous observations of VIP and CGRP immunoreactivities in child sympathetic ganglia (Roudenok et al. 1999; Roudenok 2000). It was also noted that VIP-IR nerve cells were found as a rule in small clusters and close to blood vessels (Fig. 1 d). In contrast, CGRP-IR neurons were solitary and fewer in number (Fig. 2 d).

Discussion

The results of the present investigation for the first time demonstrate parallel upregulation of vasoactive intestinal polypeptide and calcitonin gene-related peptide expression in stellate ganglia of children with different forms of congenital cardiovascular lesions. In the literature there is no data concerning the dynamics of changes in regulatory neuropeptide synthesis in sympathetic ganglia of humans with heart and vascular abnormalities, however, some experimental studies using animal and cell models have shown similar effects of hypoxia on metabolism and expression of regulatory neuropeptides. Ambalavanan et al. (1999) observed hypoxia-induced release of peptide growth factor in arterial smooth muscle cells. Schmitt et al. (1999) investigated changes in neuropeptide expression using the MX-1 tumor cell line exposed to hypoxia. Peyronnet et al. (1999) revealed an increase in the density of neuronal cell bodies immunostained for VIP in superior cervical and stellate ganglia of rats after experimental long-term hypoxia. Experimental coronary artery occlusion also causes a long-term increase in plasma VIP concentrations that decreases after reperfusion (Gyongyosi et al. 1997). Moreover, a high level expression of VIP and CGRP has been shown in human fetal sympathetic ganglia-natural hypoxia model. In fact, the present investigation revealed not only upregulation in synthesis of both neuropeptides but also similar quantitative proportions of VIP- and CGRP-IR neurons while in human prenatal sympathetic ganglia, VIP-IR nerve cells were more numerous.

The functional implication for the increase in vasoactive intestinal polypeptide and calcitonin gene-related peptide expression in sympathetic principal ganglionic neurons of children with congenital cardiovascular lesions is a matter for discussion. In addition to classic neurotransmitters these neuropeptides have potent effects on cardiac functions, including positive chronotropic and inotropic effects as well as long-lasting vasodilator effects on the coronary arteries (Wharton and Gulbenkian 1987). Besides, the neuropeptides are involved in other important physiological functions, acting as neuromodulators or trophic and inductor agents in developing and mature nerve and somatic cells (Burnstock 1982; Pincus et al. 1994; Salo et al. 1995; Klimaschewski 1997). Recent experimental studies demonstrated the role of some neuropeptides in regulation of endothelial function and angiogenesis (Zukowska-Grojec et al. 1998; Schmitt et al. 1999). Taking this information into consideration, it has been suggested that the activation of VIP and CGRP synthesis in stellate ganglia of children with congenital cardiovascular lesions is a compensatory reaction of principal ganglionic neurons providing a correspondence to congenital pathology of heart contractility regulation as well as trophic influence on the ischemic myocardium and inductor effect on its angiogenesis. Hypoxia is the main inducing factor for the upregulation of vasoactive intestinal polypeptide and calcitonin gene-related peptide expression in sympathetic neurons. Whether this increase in neuropeptide synthesis by stellate ganglia neurons, the main source of heart sympathetic innervation, is a reflection of general hypoxia which accompanies the cardiovas-

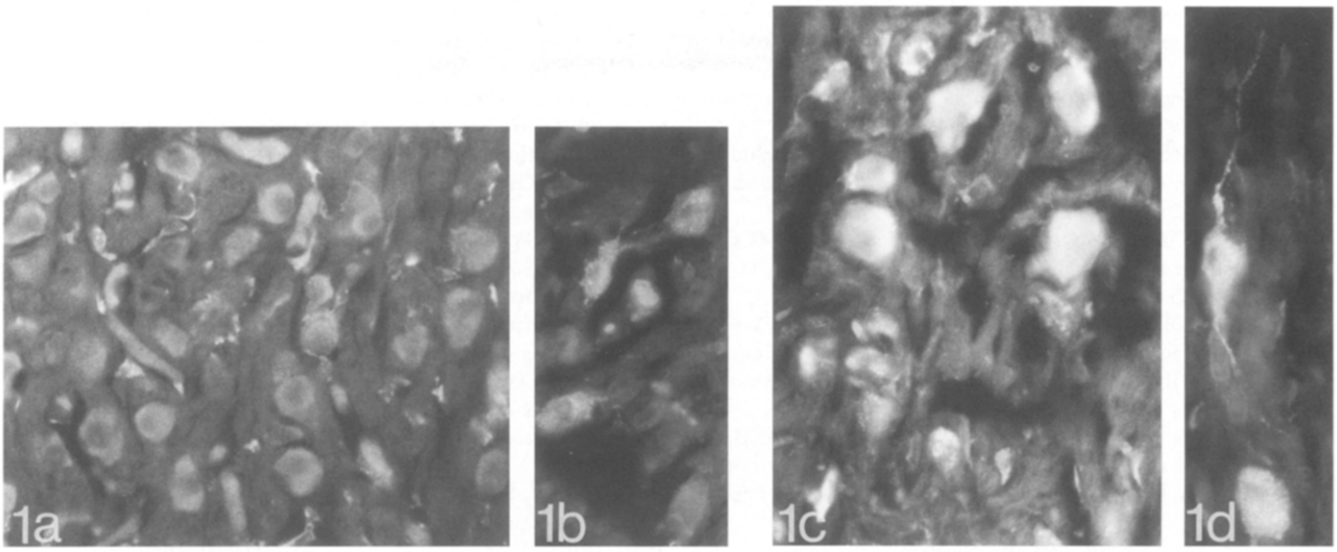

Fig. 1. Abundant VIP immunoreactive nerve cells in stellate ganglia of a neonate with Tetralogy of Fallot (a) and an infant with aortal stenosis (c). VIP positive neurons in control ganglia of a neonate (b) and an infant (d) ×400

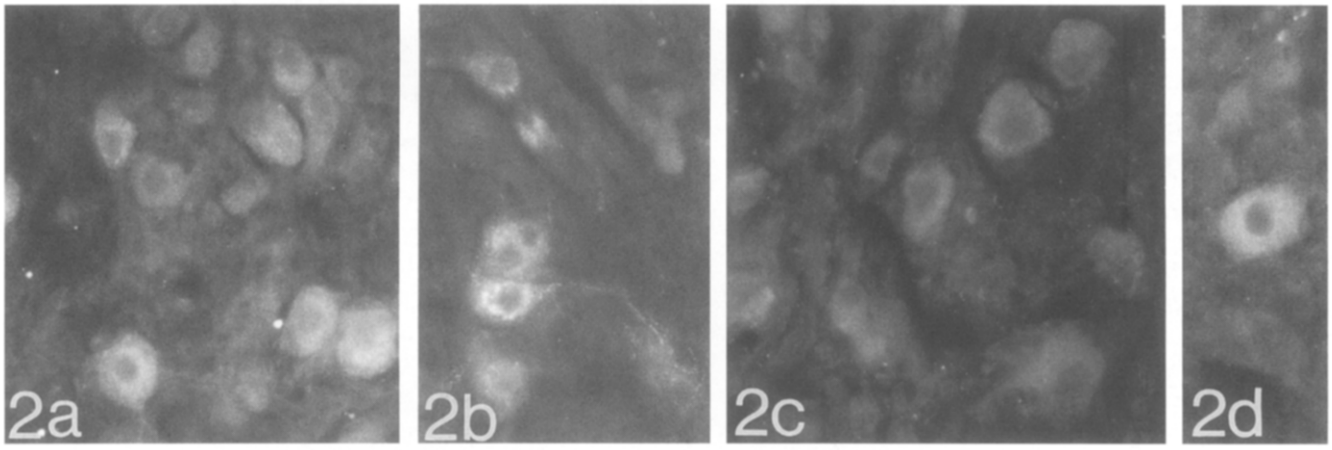

Fig. 2. Numerous CGRP-IR neurons in stellate ganglia of a neonate with hypoplastic left ventriculus (a) and an infant with aortal stenosis (c). CGRP-IR structures in control ganglia of a neonate (b) and an infant (d) ×400

cular lesions, or hypoxia of the heart itself is a question for futher clarification.

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