Histology of rat aorticopulmonary septum

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We *investigated the aorticopulmonary septum of rats for the presence of special cell types or structures. We used histological methods such as argentaffin reaction. We observed a number of solitary neuroendocrine cells, numerous mast cells and brown adipocytes in the septum. We concluded that these cells may have a physiological role in the functions of the cardiovascular system of the rats. [Turk J Med Res 1995, 13(4):131-135]*

Key Words: Aorticopulmonary septum, Neural crest

It is well known that aorticopulmonary septum is a derivative of neural crest (1,2). Bilateral removal of neural crest population prior to migration causes malformation of the septum resulting in common arterial outflow channels or transposition of the great vessels **(3).** Embryonic origin of aorticopulmonary septum has led us to hypothesize that some of the cells of the aorticopulmonary septum might have the capacity to express a special cell phenotype. To test this hypothesis we investigated the aorticopulmonary septum of the rats for the presence of such cells under light microscope.

MATERIALS AND METHODS

Twelve adult male rats weighing 150-250 gr were used in the experiment. The animals were fixed by perfusion through the left ventricle with 4% paraformaldehyde in phosphate buffer under ether anesthesia. All of the animals were grossly normal at the time of perfusion. After fixation, aorticopulmonary septae together with the related walls of the vessels were promptly removed and The Masson-Hamperl argentaffin reaction in the modification of Singh (1964) was used for demonstrating the neuroendocrine cells (4). Moreover, semi-thin plastic sections were prepared from the tissue samples after fixation in 1% osmium tetroxide to show the presence of adipose tissue. These sections were then stained with 1% methylene blue and Azure II for demonstrating the metachromatic reaction of mast cells. For each method, ten different sections from the blocks were investigated by two observers and the photographs were taken under an Olympus BHS-F2 light microscope.

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RESULTS

Silver Staining: Silver staining produced black deposits in a number of cells in the loose connective tissue of aorticopulmonary septum (Figure 1). Cells with intense silver deposits were also visualized in the tunica adventitial but no positive silver-staining cells could be found in the arterial walls (Figure 2). The cells were large oval, round or pyramidal. No argentaffin cell process or segment of nerve fibres could be visualized. The silver deposition were so intense that the individual granules were not visible clearly.

Plastic Sectioning: Plastic sections revealed a number of mast cells and lobules of brown fat in the loose connective tissue of the septum. Mast cells were observed to have larger and more dense granules than those of the neuroendocrine cells (Figure 3). Mast cells have round or oval outlines and many dark purple cytoplasmic granules. The granules nearly overlay the nuclei which were situated eccentrically. A large area in **the** aorticopulmonary septum was occupied by brown adipose tissue (Figure 4). Brown fat was organized into lobules of cells that were made up of adipocytes, capillaries, nerves, and connective tissue. These were surrounded by a thin fibrosus capsule. The cells were polygonal in shape, with a mixture of multilocular and unilocular cells. The unilocular

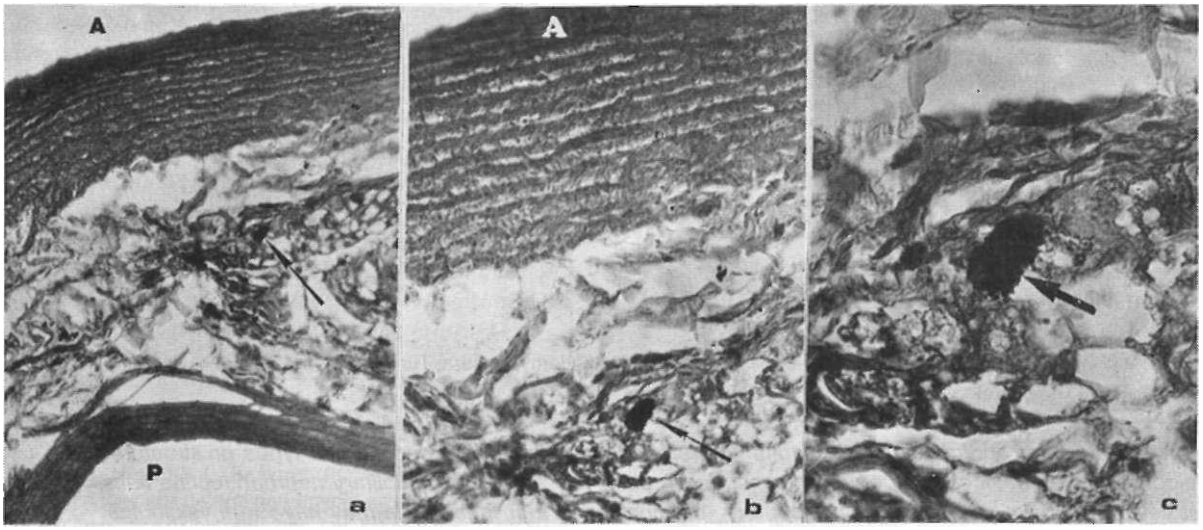

Figure 1. Three photographs with different magnifications of a solitary neuroendocrine cell (arrow) in the rat aorticopulmonary septum as demonstrated by silver impregnation. Note the intense black silver deposition in the cytoplasm. A: Aorta, P: Pulmonary trunk, a: x240, b:x480, c::x1200.

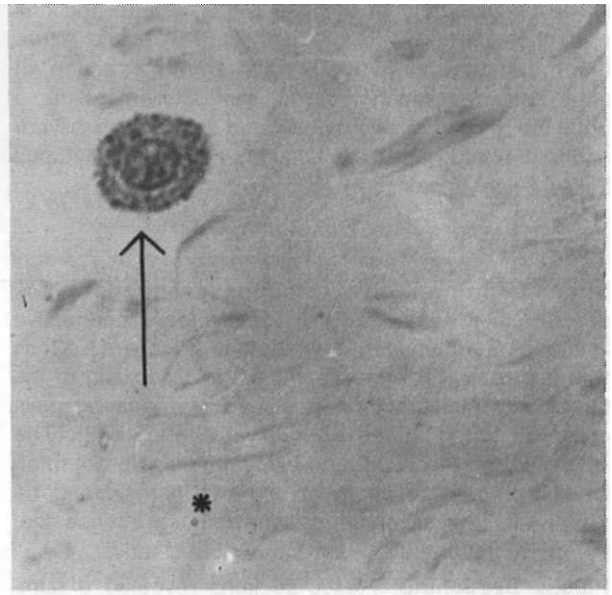

Figure 2. Two photographs of a solitary neuroendocrine cell (arrow) in the tunica adventitia of the rat pulmonary trunk as demonstrated by silver impregnation. *; Loose connective tissue, P: Pulmonary trunk, a: x480, b: x1200.

cells are indistinguishable from the mature signet ring cell type white adipocytes. The multilocular and unilocular appearance of the adipocytes are demonstrated in Figure **5.**

DISCUSSION

Neural crest cells could be recognized in the aorticopulmonary septum and in the tunica media of the

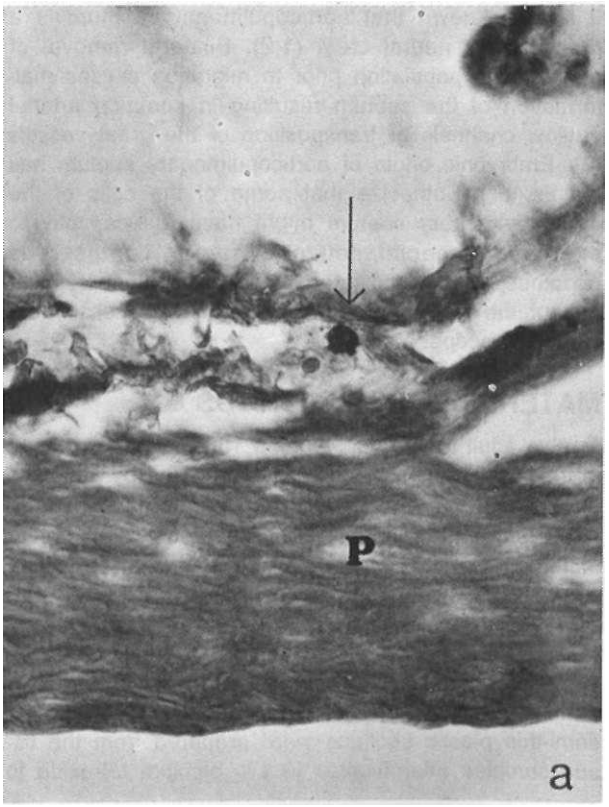

Figure 2a.

aorta and pulmonary trunk (3). These neural crest cells could play any of several roles in septal formation including the formation of an essential cell type or matrix component or the organization of mesodermal

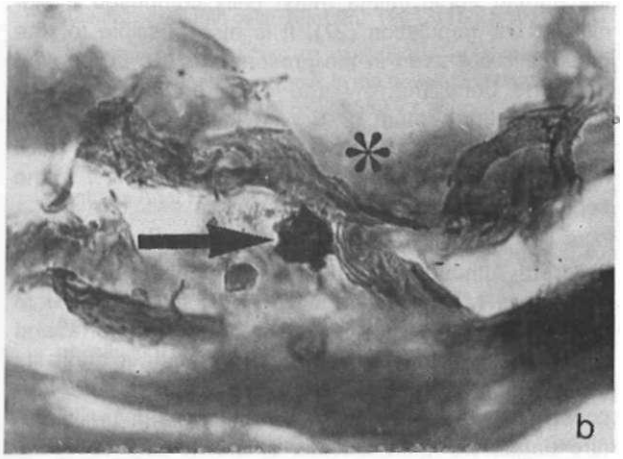

Figure 2b.

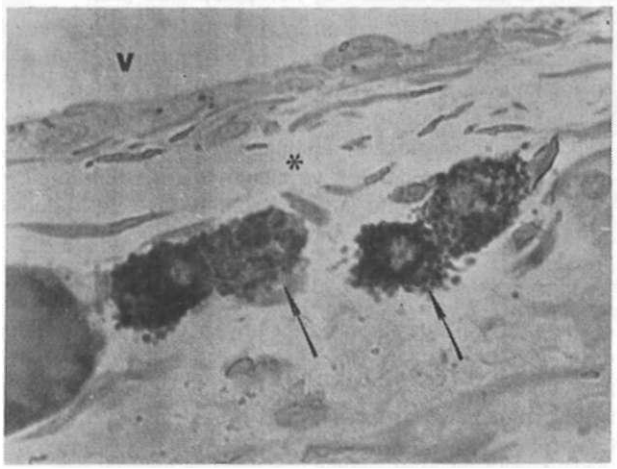

Figure 3. Mast cells (arrows) in the septum have round or oval outlines and many dark purple cytoplasmic granules. The granules stain metachromatically with toluidine blue. V: Vena Asterisk: Loose connective tissue, Plastic section, x1200.

components. It has been shown that one of the features of the ec'._<mesenchyme in the aorticopulmonary septum that distinguishes it from the contiguous non-neural crest mesenchyme is the assumption of an elastogenic phenotype (5). It was also reported that the aorticopulmonary septal cells expressed smooth muscle cell phenotype during the process of septation (6). Similarly, neural crest cells could form other essential cell types such as neuroendocrine cells in the septum.

Neuroendocrine cells according to Fujita (7), are endocrine and sensory cells that share structural, functional, and metabolic features with neurons and that produce substances identical with or related to neurohormones and neurotransmitters. The term "paraneuron" also began to be applied to these cells by many authors (8). They respiratory and gastrointestinal tracts are extensively populated by a

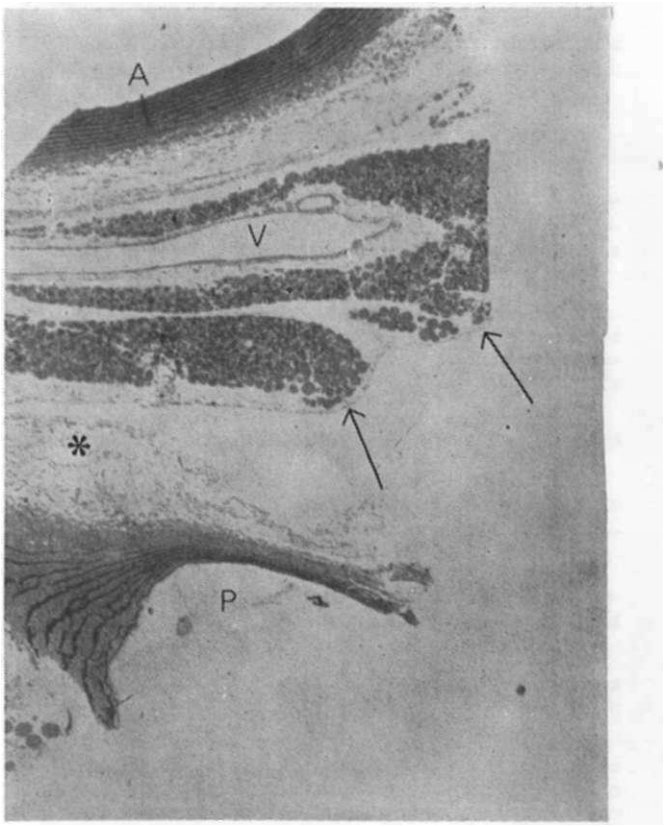

Figure 4. A photomicrograph of brown fat which is organized into lobules (arrows) surrounded by a thin fibrous capsule. A: Aorta, P: Pulmonary trunk, V: Vena, Asterisk: Loose connective tissue, Plastic section, x50.

heterogeneous collection of these cells (9,10). Moreover, as defined by their argentaffinity or argyrophilia they have also been demonstrated in the skin (11), breast (12), thymus (13), urinary bladder (14), prostat (15) and in the cervix (16). The present study demonstrates for the first time that, also the aorticopulmonary septum of rat contains a number of solitary neuroendocrine cells. We could not find any literature data about the solitary neuroendocrine cells in aorticopulmonary septum. But there are several reports showing the presence of aorticopulmonary paraganglia in a number of animals (17-19). While paraganglia are characterized by clusters of cells; the neuroendocrine cells observed in the present study were positioned individually and not as a group. Grimelius (1968) demonstrated that, the argentaffin reactions of neuroendocrine cells are due to the presence of serotonin (20). The result of the present study is in agreement with that of Grimelius. However, immunocytochemical studies are necessary to assess the contents of the granules.

The solitary neuroendocrine cells in aorticopulmonary septum may show similar phenotype with those found within the lamina propria of the gastro-

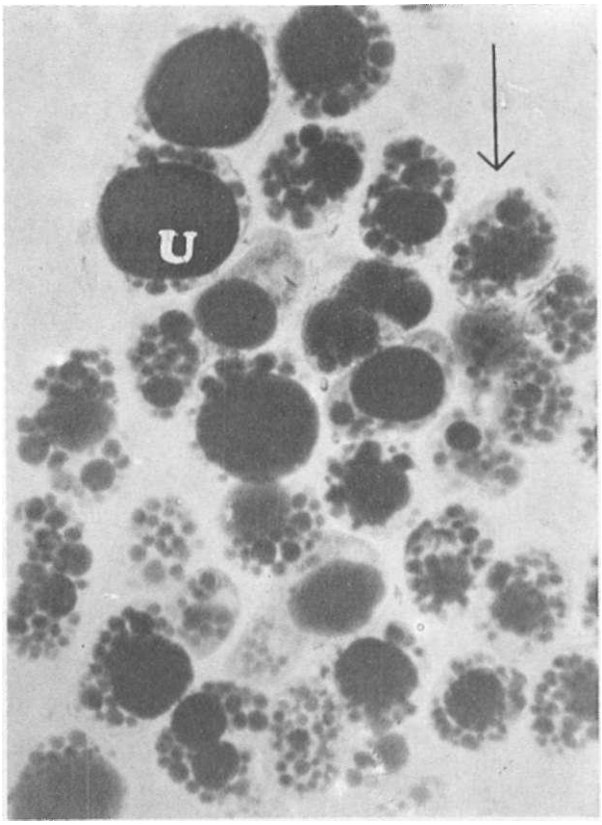

Figure 5. A photomicrograph of brown adipoj/i. 'v Y>~ aorticopulmonary septum. Brown adipocytes are observed witha mixture of multilocular (arrow) and unilocular (U) cells. The unilocular cells are indistinguishable histologically from the mature signet ring cell type white adipocytes. Plastic section, x1200.

intestinal tract (21). The neuroendocrine cells within the lamina propria were reported to have no attachment to the overlying epithelium and surrounded by Schwann cells and unmyelinated nerve fibers. However, similar nerve fibres or Schwann cells close to the neuroendocrine cells were not observed in the present study. It was reported that, neuroendocrine cells could also secrete via the blood-stream and influence more distant areas of the region or other organ systems such as the central nervous system (22). The results of the present study may also indicate a similar function.

Mast cells tend to be found in association with lymphoid follicles, in the walls of arteries, and adjacent to the endothelial cells of sinusoids and the endosteal cells of bone trabeculae (23). It is now known that the hematopoietic stem cells generate morphologically unrecognizable progenitors of mast cells within the bone marrow (24) and that the most mature ones of these cells enter the blood (25,26). The circulating cells, which still lack mast cell granules, migrate into the tissues where they proliferate and mature into mast cells. It was reported that, among the blood-borne cells in the human aortic intima, mast cells composed a significant cell population (27). It is also possible for the mast cells observed in the present study to be derived from the bone marrow and migrated into the aorticopulmonary septum (28). With their vasoactive contents and perivascular localization, septal mast cells might be involved in the regulation of blood flow of the septum (29).

Brown fat is most widely distributed in young children. Although it gradually disappears from most sites over the next several decades, it was found to persist around the kidneys, adrenals, and aorta and within the mediastinum and neck throughout adult life (30). The presence of brown adipocytes in the aorticopulmonary septum is in agreement with the literature data. The main function of brown adipose tissue ise heat production (31). The production of heat is closely related to the active sympathetic innervation of brown fat and stimulation by norepinephrine (32).

The physiologic importance of the loose connective tissue between the aorta and the pulmonary trunk is not known. But this connective tissue may function in mechanical support, storage of energy reserves in adipose cells, protection against infection, and repair - 'er injury. Loose connective tissue, with its abundant, -jhly hydrated ground substance, may also be important in the septum since the mobility of the part is ad-/antageous.

It is possible that some of the cells demonstrated in the present study could be derived from the neural crest (3,5,6,28). Any discussion about the possible functional role or roles these cells may play would be highly speculative at this stage. We suggest that these cells may act in a variety of complex ways to regulate rat caridovascular function through integration and modulation of diverse pathways.

Ratlarda aortikopulmoner septum histolojisi

Raflarda aortikopulmoner septumu, özel bir hücre veya yapı varlığı yönüyle incelendi. Bu amaçla, argehtafin reksiyonu gibi metodlar kullanıldı. Septum kesitlerinde, birkaç soliter nöroendokrin hücre, mast hücreleri ve kahverengi yağ hücreleri gözlemlendi ve bu hücrelerin ratlarda kardiyovasküler fonksiyonlar için önemli olabileceği kanısına varidi. [TurkJMedRes 1995; 13(4):131-135]

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