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Increased levels of somatostatin in rat ankles with adjuvant arthritis

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Abstract Levels of somatostatin were investigated in the ankles and spinal cords of rats suffering from acute and chronic adjuvant arthritis. As measured by radioimmunoassay, somatostatin showed significantly higher concentrations only in chronic arthritic ankles. No significant difference was observed in somatostatin levels between the spinal cords of normal and arthritic groups. Using immunohistochemical labeling and electron microscopy, we observed increased somatostatin labeling in the mature bone matrix, monocytes, and polymorphonuclear cells of bone marrow and macrophage-like synovial cells of chronically arthritic rats. Understanding the mechanism(s) which lead to increased somatostatin in chronic arthritic joints may result in more effective treatment methods.

Keywords Adjuvant · Arthritis · Somatostatin · Ankle · Spinal cord

Introduction

Rheumatoid arthritis represents one of the major problems in medicine. Arthritic joints characteristically display hyperplasia of the synovial cell lining and infiltration with various mononuclear cells, especially T lymphocytes [1, 2]. Proliferation and activation of synovial cells contribute to pannus formation, which in turn compromises joint function. In addition, maladaptive immune responses have been described in arthritic patients, including T cell infiltration of the synovium along with circulating rheumatoid factors [2].

Adjuvant arthritis can be induced in rats with a single intradermal injection of a suspension of heat-killed Mycobacterium butyricum, which yields symptoms in many ways similar to those of rheumatoid arthritis in humans [3]. For example, histological sections from the joints of these animals demonstrate pannus formation with mononuclear and polymorphonuclear infiltrates.

Somatostatin has been recognized as a potential anti-inflammatory drug and effectively used to reduce inflammation in arthritic patients and experimental animals [4, 5, 6]. Together with substance P (SP) and calcitonin gene-related peptide, somatostatin is present in sensory terminals of the peripheral nervous system. It has been suggested to be involved in the modulation of neurogenic inflammation through inhibition of SP release [7] and SP-induced neutrophil chemotaxis [8]. Somatostatin also affects the immune system by inhibiting B lymphocytic immunoglobulin production and by proliferation of T lymphocytes [9, 10]. Furthermore, decreases in peripheral lymphocyte number and function have been reported in a patient with somatostatinoma, which suggests that it may have immunoregulatory properties and anti-inflammatory action [11].

Recently we identified somatostatin in bone and joint tissues [12]. While several studies have shown that exogenous somatostatin is beneficial in human or "experimental" arthritis [4, 5, 6], changes in endogenous somatostatin levels during or after the development of the disease have not been studied. Therefore, this study was designed to investigate somatostatin concentrations in ankle joints and spinal cords of rats suffering from acute or chronic adjuvant arthritis using electron microscopy and radioimmunoassay (RIA).
Material and methods

The study included 30 female Lewis rats weighing 160–180 g each. The animals were given water and food pellets ad libitum and housed five to a cage at 21°C in a 12-h light-dark cycle. This experiment was approved by the Stockholm regional ethics committee.

Adjuvant arthritis

The experimental animals were divided into two groups: an acute arthritic group (n = 6), killed 12 days after inoculation with mycobacteria, and a chronic arthritic group (n = 12) killed together with the control rats (n = 12) 30 days after mycobacteria inoculation. Arthritis was induced by intradermal injection of a suspension (50 μl) of heat-killed *Mycobacterium butyricum* in paraffin oil (10 mg/ml) into the base of the tail. Controls received 50 μl paraffin oil by the same route.

Tissue preparation and immunohistochemistry

Six control and six chronic arthritic rats were anesthetized by intraperitoneal injection of sodium pentobarbital (60 mg/kg body weight) and perfused intra-arterially with phosphate buffer saline (PBS), followed by Zamboni’s buffered 4% paraformaldehyde solution containing 0.2% picric acid. The ankle joints were excised and immersed in Zamboni’s fixative for 2 days at +4°C. The specimens were demineralized in a 4% ethylenediaminetetra-acetic acid (EDTA) solution at pH 7.3 for 3 weeks. Subsequently the bones were rinsed for at least 2 days in 10% sucrose in 0.1 M Sörensen’s phosphate buffer, containing sodium azide and bacitracin (Sigma, St. Louis, Mo., USA). The metaphyseal bones were infiltrated with 2.3 M sucrose and frozen in liquid nitrogen.

Sectioning was performed according to Tokuyasu [13]. Grids were placed directly on drops of 0.15-M NaCl containing 20 mM glycine for 20 min. To block nonspecific binding, the grids were incubated with 10% bovine serum albumin (BSA) (Fraction V, Sigma) in 0.1 M phosphate buffer (PB) at pH 7.4 for 30 min and subsequently incubated with the primary antibody overnight at room temperature in a humidified chamber. The sections were thoroughly washed in PB containing 0.1% BSA (PPB), and bound antibodies were detected with protein A coated with 10 nm gold (Amersham, Buckinghamshire, U.K.). The gold conjugate was incubated with the sections for 1 h, and the sections were then washed and contrasted with 0.1% uranylacetate and embedded in 2% methylcellulose.

Electron microscopy

Grids were examined with a Leo 906 electron microscope (Zeiss, Oberkochen, Germany) at 80 kV. To compare somatostatin labeling in control and chronic arthritic tissues, morphometric analysis was performed by counting gold particles over cell cytoplasm or bone matrix on printed copies of micrographs (final magnification ×49,600) taken at random. The corresponding areas were measured by point counting using a square lattice (2 cm). The values obtained represent the number of gold particles per μm².

Radioimmunoassay

Eighteen rats were used in this study (six each from control, acute arthritic, and chronic arthritic groups). The animals were killed by decapitation under ether anesthesia. The ankle joints including the distal tibial epiphysis and talus, calcaneus, and tarsal bones were collected along with the joint capsule and synovial membrane. Whole spinal cords were collected by cutting the sacralumbar joint and pushing out the spinal cord using a syringe filled with normal saline. All tissues were immediately frozen on dry ice and kept at −70°C.

For extraction, the tissues were boiled for 10 min in 10 vol of 2-M acetic acid in 4% EDTA, homogenized in a polytron for 1 min, sonicated for 30 s, boiled again for 10 min, and then centrifuged at 3000 g for 15 min. The supernatants were lyophilized and kept at −20°C until analysis. An RIA analysis was performed using the somatostatin RIA kit (Peninsula, St. Helens, U.K.) as described by the manufacturer and proteins were measured according to the method of Lowry [14].

Statistical analysis

Comparisons of somatostatin levels in different tissues were measured by one-way analysis of variance (ANOVA). Post hoc tests were performed using Fisher’s protected least significant difference (LSD) analysis. Comparisons of the numbers of gold particles representing somatostatin labeling in control and chronic arthritic bone matrix, monocyte, polymorph nucleated, and synovial cells were performed with the unpaired Student’s *t*-test.

Results

In rats inoculated with mycobacteria, signs of ankle joint inflammation were apparent on day 10 and persisted until the end of the experiment. Histological analysis (hematoxylin-eosin staining) and radiographic changes of the inflamed ankles on day 30 postinoculation with mycobacteria confirmed the occurrence of inflammatory damage.

Using immunohistochemical electron microscopic techniques, we compared the labeling of somatostatin in the chronic arthritic and control bone matrices (Fig. 1a, b) and the cytoplasm of bone marrow monocyte (Fig. 1c, d), polymorphonuclear (Fig. 1e, f), and synovial cells (Fig. 1g, h). These levels were significantly higher than control levels (*P* = 0.0001) in the arthritic bone matrix (*P* = 0.0001; Fig. 2a) and in bone marrow monocytes (*P* = 0.0057; Fig. 2b). In bone marrow polymorphs, a significant increase in labeling (*P* = 0.0016) was observed in the chronic arthritic group (Fig. 2). In both arthritic and control tissues, somatostatin labeling was detected only in macrophage-like synovial cells but not in fibroblast-like cells. Significantly higher labeling (*P* = 0.0001) was observed in the arthritic macrophage-like synovial cells (Fig. 2d).

A significant increase (*P* < 0.05) in somatostatin concentration (measured in pmol/g protein) was found in ankle joints of the chronic group compared to the control and acute groups (Fig. 3a). No significant difference was detected between the control and acute groups. Spinal cord concentrations did not differ between groups (Fig. 3b).

Discussion

The interaction of the nervous, endocrine, and immune systems may play a major role in the pathophysiology of adjuvant and rheumatoid arthritis [1, 2]. The release of neuropeptides, including SP, somatostatin, and calcitonin gene-related peptide, from peripheral nerve endings