Expression of galanin and galanin-receptors in acute and chronic adjuvant arthritis.

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Introduction: Galanin, a 29-amino acid peptide, is widely distributed in the central and peripheral nervous systems as well as in several peripheral regions. Numerous reports suggest a significant role of Galanin (GAL) in inflammation and pain in musculoskeletal disorders. However, the peripheral distribution of GAL and its receptors in bone and joint tissues remains unclear. GAL significantly up regulated during inflammation, exerts its effect by three galanin receptors, GALR1, GALR2 and GALR3. Both pro and anti-nociceptive role of GAL has been observed in the spinal cord during inflammation. GALR2 is suggested to have an excitatory role in the inflammatory pain while its anti-nociceptive effect is suggested through GALR1.

The current knowledge about the distribution of galanin receptors in bone and joint tissues is quite limited. The aim is to study the expression of GAL and its receptors (GALR1 and GALR2) in central and peripheral nervous system and in the peripheral tissues during acute and chronic inflammatory conditions.

Materials and methods: The study included 32 female Lewis rats, body weight 200-230 g. The animals were housed according to Karolinska Institutes protocol. All experiments were approved by the Ethical Committee for Animal Research, Stockholm. Arthritis was induced in 30 rats by heat killed Mycobacterium butyricum injected at the base of tail. Signs of inflammation in the ankle joints appear about 13 days after inoculation with mycobacteria. Measurements were taken from day 21 and day 28 as acute and chronic arthritis.

Immunohistochemistry: Twelve rats (6 controls+6 chronic arthritic) were anaesthetised with sodium pentobarbitone and perfused with 0.01 mol/l phosphate buffered saline, followed by Zamboni’s fixative. Bilateral ankle joints were dissected and immersed in the same fixative for two days before subjecting to demineralization in a 4% EDTA. Spinal cord, DRG and demineralized ankle joints, were soaked in 20% sucrose until sectioning. The sections 15 μm were cut on a Leitz cryostat, mounted directly on SuperFrost/Plus slides and immunostained with antiserum to GAL and PGP (1:10000) before incubation with the fluorochrome Cy3-conjugated avidin (1:10000) for visualization of the immunoreaction.

Real-time qPCR: Total RNA was extracted from spinal cord, DRG (L2, L3) and ankle joints from control, acute and chronic arthritic rats, six in each group. Samples were immediately frozen in liquid nitrogen. Frozen samples were dismembranated using Mikro-Dismembrator and total RNA was extracted using the RNeasy MiniKit (Qiagen). Quantification assays were performed to detect the relative expression of GALR1 and GALR2 mRNA with TaqMan 1X Universal PCR Master Mix (Applied Biosystems, Roche) and run on ABI Prism 7700 Sequence Detection System. The β-actin and B2M genes were amplified as endogenous reference and the relative expression of GALR1 and GALR2 from each sample was normalized by the mean value of β-actin and B2M.

Results: Our IHC showed galanin labelling in all the studied tissues. In the DRG, few small to medium sized neurons were immunostained in the healthy rats as compared to arthritic rats where the number of positively stained neurons was significantly high (Fig 1 A, B). GAL-positive nerve fibres were occasionally present in the periosteum and synovial membrane of the ankle joint in healthy controls as thin, varicose, nonvascular nerve terminals Fig 1 C. Dramatic up regulation in GAL expression was observed in the adjuvant induced rat ankles, in synovial membrane, periosteum as well as in bone and in connective tissues. In the synovium labeling was observed in thin, varicose nerve terminals and in the Periosteum mainly myelinated nerve fibres positively stained with GAL were observed Fig 1 D. Extensive GAL labelling was observed in the SC especially in the dorsal horn during the inflammation (results not shown).

Real time PCR analysis showed differential GALR1 and GALR2 expression in all tissues under acute and chronic arthritic stages. GALR1 expression was significantly increased in the SC during inflammation. Contrary to it, GALR1 expression was significantly decreased in DRG and in ankle joint, during inflammation except under acute arthritis in the DRG. GALR2 expression was significantly decreased in SC, DRG and in ankle joint during inflammation. In the DRG, GALR2 expression was highest during normal conditions when compared to all studied tissues which were significantly decreased during inflammation Fig 2.

Discussion: This study clearly demonstrates the role of GAL in the mediation of inflammation and inflammatory pain the peripheral bone and joint tissues. Our immunohistochemical studies demonstrate that GAL is predominantly present in nerve fibres in the synovium, periosteum, and in bone. We also confirm the presence of galanin receptors in the ankle joint as well as in the corresponding DRG and in the SC. It is a common clinico-pathological observation that structures like periosteum, synovium, capsule and muscles are pain sensitive and prone to inflammation. Taken together these findings, we speculate that galanin in a paracrine/autocrine fashion participates in pain transmission during inflammatory disorders of musculoskeletal system like arthritis.

Further, our quantitative PCR results indicating differential distribution and expression of galanin receptors in the SC, explain the contrasting effects of GAL as both pro- and anti-nociceptive as observed by previous studies.

Our study explains the presence of multiple receptor subtypes, density and different signalling pathways of galanin in bone and joint tissues which should be consider during pathological conditions.