



# Analysis Of Substance P Receptor mRNA in Periosteum and Capsular Tissues of Adjuvant Arthritic Rat using Hemi-nested RT-PCR

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## Objective:

To establish Reverse transcriptase Polymerase Chain Reaction (RT-PCR) on Bone and joint tissue of adjuvant arthritic rat and to find expression of Neuropeptide receptor SP.

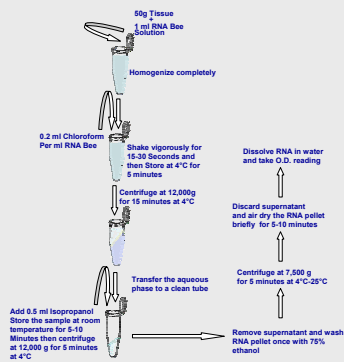
## Introduction

- Substance P (SP) is a neuropeptide considered to function as a neurotransmitter or modulator in the central and peripheral nervous system (1)
- Regulation of SP receptor (SPR) synthesis most likely occurs at the level of transcription. SPR mRNA is widely but unevenly distributed throughout the rat central nervous system and periphery. The highest level of SPR mRNA is in the urinary bladder (2). SP receptor's RNA is normally below detectable level (3,4).
- There is evidence that SP mediates neurogenic inflammation in adjuvant arthritis and contributes to the severity of the arthritis (5).
- The mRNA level in tissues of adjuvant arthritis has not yet been reported in the literature. This is the first report that describes the method for the analysis of SPR mRNA in adjuvant arthritic rats.

## Material And Method

Arthritis was induced by intra dermal injection of 50 ul of heat killed *M. butyricum* in paraffin oil (50mg/ml w/v) into the base of the tail. Following induction of arthritis rats were dissected and samples were collected in the tube into liquid nitrogen and stored in -70°C.

### RNA Extraction (By RNA Bee Reagent)



### Reverse Transcriptase-RNA Polymerase Chain Reaction

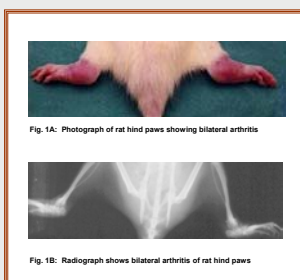
#### Reverse Transcription:

- cDNA was synthesized by using first strand cDNA synthesis kit from Roche, by using approximately 1 ug of total RNA, 20 U of avian myeloblastosis virus reverse transcriptase, 50 U of RNase inhibitor, 1mM deoxynucleotide triphosphates (dNTPs), 5mM MgCl<sub>2</sub> and 1.6 ug oligo dT primers per 20 ul reaction for 10 minutes at 25°C and then at 42°C for 60 minutes followed by incubation for 5 minutes at 99°C.

#### Polymerase Chain Reaction:

- PCR was performed on 10% of the cDNA using a final concentration of 1.6 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 25 pM of substance P receptor forward and external reverse primers and 2 units of ampli taq DNA polymerase per 50-ul reaction. RNA from S.Cord was used as a positive control.
- Thermal cycling program was as follow: Denaturation was at 94°C for 40 seconds, annealing was at 58°C for 40 seconds and extension was at 72°C for 1 minute and 30 seconds for 40 cycles.
- For hemi-nested amplification, same reagent concentrations and thermal cycling program was used except using internal reverse primer with same forward primer.
- PCR products were analyzed by electrophoresis through 2% agarose gels and viewed under UV light after ethidium bromide staining.
- PCR with rat β-actin primers was performed on 10% of the cDNA using a final concentration of 1.6 mM MgCl<sub>2</sub>, 0.2 mM dNTPs, 25 pM of each primer and 2 unit of taq DNA polymerase per 50-ul reaction. RNA from Spinal Cord was used as a positive control.
- Thermal cycling program was as follow: Denaturation was at 95°C for 60 seconds, annealing was at 61°C for 60 seconds and extension was at 72°C for 90 seconds for 35 cycles.

## Results:

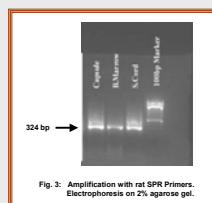
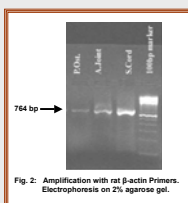
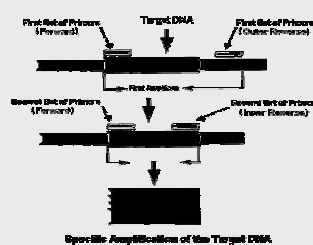


Primers sequences used for Amplification of cDNA:

Rat beta actin primer:  
 Forward 5'-TTGTAACCAACTGGGACGATATGG-3'  
 Reverse 5'-GATCTTGATCTTCATGCTAGG-3'  
 Expected length of PCR product is 764 bp.

Rat Substance P receptor Primers:  
 Forward 5'-CCTCTACTGGTGATCGGCTACG-3'  
 Reverse (External) 5'-CCTCTACTGGTGATCGGCTACG-3'  
 Reverse (Internal) 5'-TGTGCTGGAGGATCGGGTGG-3'  
 Expected lengths of PCR products are:  
 With external reverse primer 561 bp  
 With internal reverse primer 324 bp

#### Schematic diagram of hemi-nested amplification



## Conclusion:

We have developed and tested a hemi-nested RT-PCR assay for the detection of SPR mRNA in periosteum and capsular tissues of adjuvant arthritic rats.

Our preliminary data show higher expression of SPR mRNA in arthritic rats compared to normal controls. In future we plan to use this test for further SPR studies in adjuvant arthritis and anti-inflammatory rat models.

## References:

- B. Pernow, Pharmacol. Rev. 35, 85 (1983)
- Hershey, A. D., and Krause, J. E. (1990) Science 247, 958-962.
- Akifumi Togari, Michtsugu Arai, Shigeki Mizutani, Shigeru Mizutani, Yasuko Koshihara, Toshiharu Nagatsu. Expression of mRNAs for neuropeptide receptors and β- adrenergic receptors in human osteoblasts and human osteogenic sarcoma cell. Neuroscience letters 233 (1997) 125-128.
- Bjurholm A, Kreicbergs A, Schultzberg M, and Lerner U.H. Neuroendocrine regulation of cyclic AMP formation in osteoblastic cell lines (UMR-106-01, ROS 17/2.8, MC3T3-E1, and Saos-2) and primary bone cells. J. Bone Miner Res.7(1992) 1011-1019
- Levine JD, Clark R, Devor M, Helms C, Moskowitz MA, Basbaum AI. Intra-neuronal substance P contributes to the severity of experimental arthritis. Science. 1984 Nov 2;226(4674):547-9.