THE EFFECTS OF TACHYKININS AND THEIR METABOLITES ON ARTICULAR CARTILAGE CHONDROCYTE AND SYNOVIOCYTE FUNCTION

by

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THESIS SUMMARY

The effect of mammalian tachykinins and the metabolites of substance P (SP) have been studied on bovine articular chondrocyte and synoviocyte function.

Chondrocytes were isolated from articular cartilage and grown in monolayer culture *in vitro*. Cartilage slices were also used *in vitro* in some experiments. Both human and bovine synovial cells were cultured from explant cultures *in vitro*.

Bovine articular chondrocytes in monolayer culture failed to alter proteoglycan synthesis as measured by CPC precipitation of $^{35}SO_4^=$ labelled glycosaminoglycans in response to SP, neurokinin A (NKA) or neurokinin B (NKB). Furthermore the N-terminal, SP-(1-4), SP-(1-6) and the C-terminal SP-(7-11) fragment of SP also failed to alter proteoglycan synthesis. The molecular size of proteoglycan present in the cell layer was assessed by column chromatography following exposure to SP. No alteration of proteoglycan monomer was observed following exposure to SP. Similar profiles were obtained from proteoglycans released into the cell supernatants. Total protein production was also unaffected by SP.

However the C-terminal fragment of SP, SP-(7-11) significantly increased prostaglandin E_2 (PGE_2) production. Intact tachykinins and the N-terminal fragments of SP did not alter PGE_2 production. SP-(7-11) was found to increase PGE_2 production by approximately 60% at 10^{-4} M. This effect was completely inhibited by the cycloxygenase inhibitor, indomethacin. Furthermore spontaneous PGE_2 production was unaffected by extracellular EGTA. However the stimulatory effect of SP-(7-11) could be completely inhibited when chondrocytes were cultured in the presence of extracellular EGTA. The non-peptide NK-1 receptor antagonist, CP-96,345 was found to inhibit the stimulatory effect of SP-(7-11) on PGE_2 production. PGE_2 production was inhibited by 60-70% when SP-(7-11) was incubated in the presence of 10^{-4} M CP-96, 345.

Bovine articular chondrocytes which had been previously exposed to hr IL-1α had an enhanced response to SP-(7-11), suggesting that hr IL-1α augments the effect of SP-(7-11).

Bovine articular chondrocytes also increase the production of matrix metalloproteinase I (MMP-I) in response to SP-(7-11). This response was maximal at 10^{-4} M SP-(7-11) where MMP-I synthesis was approximately five times that of control and could be blocked by cycloheximide. SP did not alter MMP-I synthesis. In response to SP-(7-11) chondrocytes were found to secrete latent MMP-I. Tissue inhibitor of metalloproteinase (TIMP) production was not induced by SP-(7-11).

Intracellular calcium concentration $[Ca^{2+}]_i$ was measured using the fluorescent dye FURA-2AM. Chondrocytes loaded with FURA-2AM also respond to SP-(7-11). This response was maximal at 10 μM and the change in $[Ca^{2+}]_i$ was approximately 140 nM. This effect could be completely blocked by extracellular EGTA. Following the initial stimulation with SP-(7-11), subsequent stimulation with SP-(7-11) had no further effect suggesting desensitization of this mechanism.

Both bovine and human rheumatoid synoviocytes cultured from explants failed to alter PGE_2 production in response to tachykinins or the fragments of SP. These studies demonstrated that SP metabolism by the metalloendopeptidase, neutral endopeptidase (NEP), CALLA or CD10 antigen into SP-(7-11) is of biological significance.

The major finding from this work has been that the metabolism of SP and the subsequent production of SP-(7-11) is important in regulating the biological activity of SP on chondrocytes in the synovial joint.