

Effects of capsaicin on the metabolism of rheumatoid arthritis synoviocytes in vitro

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Abstract

The effects of capsaicin, the ingredient of hot pepper, on rheumatoid arthritis synoviocytes have been studied. Capsaicin was shown to have a direct action on the metabolism of synovial cells. Thus at 10^{-6} mol/l cell proliferation was noted while at 10^{-8} mol/l and at higher doses DNA synthesis was restored to the control level. Capsaicin at both doses induced an increase in the synthesis of collagenase and at the lower concentration (10^{-8} mol/l) only of prostaglandins.

These results indicate that the different effects of capsaicin on cellular proliferation and on metabolic activities are dependent on dose. The responses seen in rheumatoid arthritis synoviocytes in vitro might not be mediated by tachykinins if the synovial tissue is still able to produce neuropeptides in the absence of neuronal afferents. These results suggest that capsaicin, in addition to its direct action on the afferent nervous fibres and the consequent release of tachykinins, may also have a direct action on the cells. The mechanisms by which capsaicin stimulates DNA synthesis and production of collagenase and prostaglandin E_2 , in a manner dependent on dose, remain to be determined.

Rheumatoid arthritis (RA) is a disease characterised by chronic synovial inflammation leading to cartilage destruction, which is believed to result from the production of metabolites of arachidonic acid and proteases.^{1 2}

Explants of RA synovial tissue are now in widespread use for in vitro culture of synoviocytes. These cultures, deprived of nervous system control, provide a useful experimental tool to study the cellular metabolism and the response to substances that may interfere with the inflammatory process.

Capsaicin, a homovanillic acid derivative (8-methyl-*N*-vanillyl-6-nonenamide), has been used as an experimental tool to investigate the functional and neurochemical characteristics of peptide containing primary sensory neurones.^{3 4} Recently, it has been used on humans for topical treatment of cluster headache,⁵ herpes zoster,⁶ and vasomotor rhinitis.⁷

Systemic administration of capsaicin to newborn rats results in an irreversible, selective degeneration of a distinct population of small size B type primary afferent neurones located in spinal and cranial sensory ganglia.⁸ Likewise, capsaicin given systematically to adult rats induces reversible damage in the same fibres.⁹ One of the most important neurotoxic responses

to capsaicin is the damage of mitochondria and the change of the nuclear shape. These modifications and the dilatation of endoplasmic reticulum and Golgi apparatus have been shown in a major subpopulation of B type neurones.^{10 11} The toxic effects of capsaicin have also been described in endothelial cells.¹²

In rats with adjuvant arthritis subcutaneous pretreatment with capsaicin has been shown to moderate synovitis¹³ and attenuate the increase in substance P content, due to arthritis, in various nervous tissues.^{13 14} Inman and coworkers showed that intra-articular capsaicin can moderate the severity of synovitis in cats.¹⁵ On the other hand, acute administration of capsaicin to adult rats results in a substantial depletion, from primary afferent fibres, of substance P and other tachykinins^{16 17} that are considered the putative mediators of neurogenic inflammation.^{18 19}

Yaksh showed that when capsaicin was given intra-articularly in vivo in the cat substance P like immunoreactivity was evoked in the synovial fluid.²⁰

It thus seems that (a) tachykinins are present in the synovial fluid, (b) they can be released by capsaicin, and (c) capsaicin treatment has beneficial effects in adjuvant arthritis. But is there a correlation between depletion of tachykinins and the beneficial effect of capsaicin? Might capsaicin have a direct action on the synovial tissue independent of its effect on afferent fibres? At present there are no published reports on the effects of capsaicin on RA synovial cell cultures.

In our study we investigated the effect of capsaicin on various metabolic functions of RA synoviocytes, studying both DNA and RNA synthesis and also the production of collagenase and prostaglandin E_2 (PGE₂).

Materials and methods

RADIOLABELLED SUBSTANCES

Methyl [³H]thymidine (specific activity 3·1 TBq/mmol, 5-[³H]uridine (specific activity 1·1 TBq/mmol), and 1-[¹⁴C]acetic anhydride (specific activity 370 MBq/mmol), all from NEN Chemicals, USA, were used.

PREPARATION OF SYNOVIAL CELLS

Synovial tissue was obtained from patients with definite RA undergoing reconstructive joint surgery.

Cultures of synovial cells were grown in Parker 199 medium (supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml

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streptomycin, and 15% fetal calf serum) (PAA, Austria), hereafter referred to as the medium. Cells were used between the fifth and the 10th passages. During experiments cells were kept in 24 or 96 well culture plates (Nunclon Delta; Nunc, Denmark). Six synoviocyte cultures were plated in quadruplicate except for the control samples (six sets). All cultures and incubations were obtained in a CO₂ incubator at 37°C in an atmosphere of 5% CO₂. The same cultures were used for each experiment.

The effect of capsaicin (Sigma Co, USA) on synoviocytes was tested at doses between 10⁻⁸ and 10⁻³ mol/l.

DNA SYNTHESIS

The mitogenic effect of capsaicin was measured according to Partsch *et al.*²¹ with [³H]thymidine as a precursor for DNA. Briefly: 2 × 10⁴ cells in 100 µl medium were seeded into microwell plates. After 24 hours the Parker 199 medium was changed and replaced by new medium. Capsaicin (10 µl) was added to obtain the final concentrations shown in the figures. After 24 hours, synoviocytes were labelled with 37 kBq [³H]thymidine and incubated for a further 18 hours. The medium was discharged, the cells were washed several times with Hanks's solution and then covered with 100 µl of 10% trichloroacetic acid for 20 minutes. Methanol (100 µl) was added, removed after 10 minutes, and the cells were dried by a stream of air. The cells were lysed in 100 µl 0.3 N NaOH for three hours at 37°C, the supernatant was well mixed, and the radioactivity in 50 µl of supernatant was measured by a Packard scintillation counter using Instagel (Packard, USA).

RNA SYNTHESIS

RNA synthesis was evaluated by increasing [³H]-uridine incorporation into confluent synovial cells according to the methods of Partsch *et al.*²¹ and Schmid *et al.*²² with some modifications. Cells (2.5 × 10⁴) were seeded in 24 well culture plates (0.5 ml medium) and kept for six days until they were confluent. The medium was changed at day 3. Confluent cells were held for one hour in 0.5 ml of a protein free medium (Hanks's solution), which was replaced by 0.5 ml of medium (Parker 199 plus 2 g/l human albumin and 1 µM uridine; Sigma Co, USA). The substances under study (50 µl) were added and the cells incubated for four hours. Cells were labelled with 37 kBq [³H]uridine and incubation continued for 18 hours. Trichloroacetic acid treatment and washing were the same as for the DNA experiments. The cells were lysed in 0.3 ml N NaOH for two hours at 37°C, the solution was diluted with 0.7 ml distilled water, and 0.9 ml solution used for the measurement of radioactivity (Instagel; Packard, USA).

COLLAGENASE

Preparation of radiolabelled collagen

Collagenase release from RA synoviocytes cultured in medium, in the presence of capsaicin,

was studied according to the method of Johnson-Wint.²³

Digestion of a ¹⁴C labelled collagen film was used in this assay. The collagen type III (calfskin; Sigma Co, USA) was labelled with the 1-[¹⁴C]acetic anhydride for two hours at 6°C (instead of one hour as indicated by Johnson-Wint). The specific activity of the radiolabelled collagen was about 11 000 dpm/mg collagen. A 3 ml aliquot of this ¹⁴C labelled collagen solution was dialysed (three times against 800 ml 0.15 M sodium phosphate puffer, pH 7.4). ¹⁴C labelled collagen solution (25 µl) was added to each well of a 96 well, flat bottom Nunclon tissue culture plate (in an angle of 45°) and treated further as described by Johnson-Wint.

Collagenase assay

Release of collagenase was measured in the medium of six RA synoviocyte cultures. Cells (10⁴) were plated in 24 well tissue culture plates, as described by Lotz *et al.*²⁴ After six days of culture the cells were washed three times with Hanks's solution and replaced by 0.5 ml medium (Parker 199) free from fetal calf serum. After the addition of 50 µl of capsaicin (final concentration as given in 'Methods') cells were incubated for a further 48 hours. The supernatants were centrifuged at 12 000 rpm and frozen at -70°C. The supernatant (500 µl) was treated with 5 µl trypsin (100 µg/ml medium, type III; Sigma, USA) for 10 minutes at 25°C to activate latent collagenase. Then 10 µl soybean trypsin inhibitor (type I-S; Sigma, USA) was added and the solution left for 20 minutes at room temperature. Portions of the solution (200 µl) were placed in the wells coated with ¹⁴C labelled collagen. After an incubation of three hours at 37°C the radioactivity in 150 µl was counted by liquid scintillation spectrometry using Instagel (Packard, USA). Fresh culture medium that had been treated in the same way was used as control. The disintegrations per minute of the control were subtracted from the mean values obtained from the synoviocyte supernatant.

PROSTAGLANDIN

The influence of capsaicin on the PGE₂ synthesis of synoviocytes was studied by the method of Lotz *et al.*²⁴ with some modifications.

Cells for culture were kept in 24 well Nunclon plates with 0.5 ml medium. Cells (2 × 10⁴) were inoculated in medium plus 15% fetal calf serum and HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid). The next day the medium was replaced by fresh medium and the cells cultivated for one week up to confluency. The medium was then changed and replaced by fresh medium containing capsaicin (50 µl). The cells were incubated for a further 48 hours and then the medium was collected and centrifuged. Cell free supernatant (450 µl) was put into polypropylene tubes and stored at -70°C for further treatment. Extraction of PGE₂ was as follows: 450 µl of the medium were brought to pH 3.5 with 0.5 N HCl. Ethyl acetate (2 ml) was added, the samples shaken for 30 seconds, and

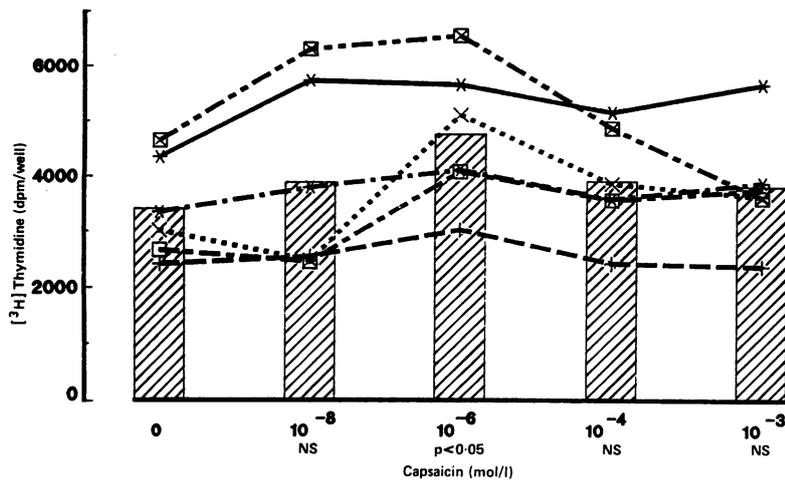


Figure 1: Thymidine incorporation into synoviocytes was stimulated by capsaicin at 10^{-6} mol/l. Capsaicin at 10^{-8} , 10^{-4} , and 10^{-3} mol/l had no significant influence on DNA synthesis in comparison with the control. The experiments were performed with six different cultures in quadruplicate.

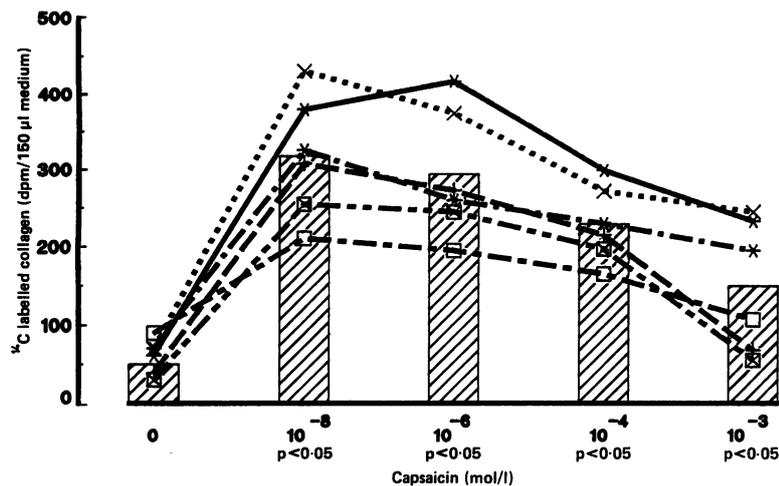


Figure 2: Collagenase synthesis induced by capsaicin at different concentrations. The production of collagenase was increased by all capsaicin doses tested in this study. The six cell cultures were each performed in quadruplicate.

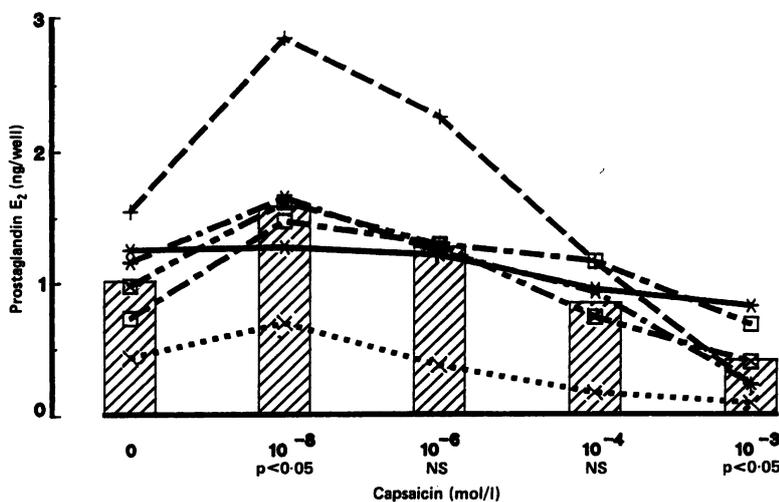


Figure 3: Synthesis of prostaglandin E₂ by rheumatoid arthritis synoviocytes, in the presence of different concentrations of capsaicin. With low doses (10^{-8} mol/l) capsaicin stimulates the release of prostaglandin E₂, whereas higher doses progressively reduce the synthesis.

centrifuged at 1500 g. From the organic phase 200 μ l were taken and evaporated to dryness. The residue was dissolved in 600 μ l distilled water and from this solution 20 μ l used in a radioimmunoassay kit (NEN Products, West Germany). The radioactivity was measured in a gammacounter. Standard PGE₂ was also added to the medium and extracted in the same way as the samples. Recovery of PGE₂ was about 96%. The counts were calculated by a standard curve and the results expressed as a percentage of the control PGE₂.

ANALYSIS OF THE RESULTS

The statistical significance of the results for the six cultures was examined with a parameter free Wilcoxon signed rank test. The course of the individual cultures and their mean values are both shown in the figures.

Results

NUCLEIC ACID SYNTHESIS

DNA synthesis

Cell proliferation was studied by measuring thymidine incorporation into synoviocytes in the presence of increasing concentrations of capsaicin (10^{-8} to 10^{-3} mol/l) (fig. 1). In comparison with baseline controls the maximal significant ($p < 0.05$) increase of thymidine incorporation was obtained with capsaicin at 10^{-6} mol/l. Concentrations of capsaicin 10^{-8} mol/l and higher did not induce a statistically significant thymidine incorporation.

RNA synthesis

The results indicate that capsaicin did not cause any statistically significant change in uridine incorporation at any of the concentrations tested.

COLLAGENASE

Each concentration of capsaicin from 10^{-8} to 10^{-3} mol/l significantly increased the release of collagenase from synoviocytes into the medium (fig 2). The highest stimulation occurred at a capsaicin concentration of 10^{-8} mol/l ($p < 0.05$). Stimulation of collagenase synthesis was less but still significant at higher doses of capsaicin also ($p < 0.05$).

PROSTAGLANDIN E₂

Figure 3 shows the results for PGE₂ production. Clearly, rising concentrations of capsaicin increased the synthesis of PGE₂ by RA synoviocytes. In comparison with controls maximal stimulation of PGE₂ production occurred at a capsaicin concentration of 10^{-8} mol/l ($p < 0.05$).

Higher concentrations caused a statistically significant progressive reduction of PGE₂ synthesis when compared with 10^{-8} mol/l. A peculiar inhibition of PGE₂ synthesis in comparison with controls was found at 10^{-3} mol/l.

Discussion

This study shows that capsaicin exerts a direct action on the metabolism of synovial cells obtained from RA. The data suggest that capsaicin can induce cell proliferation at fairly low concentrations (10^{-6} mol/l) while higher concentrations restore the cellular DNA synthesis to the level of controls. Capsaicin at low concentrations (10^{-8} mol/l) has also been shown to increase the synthesis of collagenases and prostaglandins. At higher concentrations the release of collagenases and prostaglandins into the culture medium by rheumatoid synoviocytes was reduced. These results suggest that capsaicin may have different actions on cellular proliferation and on metabolic activities. Inflamed synovial tissue is known to proliferate rapidly²⁵ and to produce high concentrations of collagenase and prostaglandins.¹

Collagenase is a major product of fibroblasts,^{2,26} and it is apparent that fibroblasts have multiple mechanisms which regulate the activity and synthesis of the enzyme.^{2,27,28}

Cultures of RA synovial tissue produce large amounts of collagenase, which can initiate breakdown of types I, II, and III interstitial collagens, thus giving this enzyme a major role in the degradation of connective tissue in both normal and pathological conditions.²

Therefore production of collagenase and PGE₂ by RA synoviocytes is of potential importance in the degradation of articular cartilage and adjacent joint structures. Indeed, PGE₂, an inflammatory lipid mediator, may have an additional role in provoking several effects, one of which might be inducing bone resorption.

Other authors have shown that capsaicin can induce PGE₂ release in homogenates of seminal vesicles.²⁹ In particular, it has been shown that capsaicin induces PGE₂ release after chronic denervation of the rabbit ear, suggesting that capsaicin may exert other effects besides those mediated through sensory nerve stimulation.³⁰

Capsaicin and its analogues deplete tachykinins in primary sensory neurones causing vasodilatation and neurogenic plasma extravasation.³¹ These effects may be due, at least in part, to the release of substance P and other tachykinins. Recently, it has been suggested that substance P activates RA synoviocytes²⁴ to produce collagenase and PGE₂²⁴ and cytokines.³²

Furthermore, other authors^{24,33,34} have suggested that neurogenic inflammation might have a role in the pathogenesis of RA. For tachykinins it is interesting to note that treatment with somatostatin, which is known to inhibit the release of substance P from peripheral terminals of primary afferent neurones,³⁵ is effective in RA³⁶ and psoriatic arthritis.^{37,38}

In our study the modifications induced by capsaicin in RA synoviocytes are, as far as we know, not mediated by tachykinins as they were found in isolated synoviocytes in the absence of neuronal tissue. We cannot, however, exclude the possibility that synoviocytes themselves might be able to produce neuropeptides. These results suggest that capsaicin, in addition to its direct action on the afferent nerve fibres with consequent release of tachykinins, also acts

directly on synovial cells. An increase of sodium influx, intracellular accumulation of NaCl, and the consequent osmotic changes have been implicated as the mechanisms responsible for the neurotoxic action of capsaicin in cultured sensory neurones.^{39,40} The mechanism by which capsaicin stimulates DNA synthesis and production of collagenase and PGE₂, however, in a manner dependent on dose, remains to be determined.

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