Neither nociceptin nor its receptor are present in human synovial fluid or tissue

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Our aim was to identify the nociceptin receptor and its endogenous ligand, nociceptin, in human peripheral tissue. Synovial tissue was obtained from 11 patients (ASA I–III, 66–84 yr) undergoing elective total knee replacement. Synovial fluid was obtained from another 10 patients (ASA I–III, 57–81 yr). Fluid was mixed with trifluoroacetic acid and the tissue with isopentone before freezing at –70°C. Nociceptin receptor identification was performed using a [3H]nociceptin binding assay and nociceptin detection by radioimmunoassay. There was no specific [3H]nociceptin binding to knee synovial tissue and radioimmunoassay did not detect nociceptin. Neither the nociceptin receptor nor nociceptin was found in human synovial tissue or fluid.

Br J Anaesth 1999; 83: 470–1

Keywords: receptors, nociceptin; pharmacology, nociceptin; surgery, orthopaedic

Accepted for publication: March 24, 1999

Cloning of the δ opioid receptor was followed rapidly by identification of cDNA for the μ and κ opioid receptors. In addition, another G-protein-coupled receptor with considerable homology to μ, δ and κ opioid receptors was also identified. This receptor was named the orphan receptor (ORL-1) and was found in areas of the brain and spinal cord involved in pain perception but did not bind classical μ, δ and κ opioid ligands with high affinity.1-3 Throughout the remainder of this manuscript, ORL-1 is referred to as the nociceptin receptor (NCR).

In 1995, a heptadecapeptide was isolated and identified as the endogenous ligand for NCR.1-3 This peptide was named orphanin FQ or nociceptin and bound to NCR with high affinity. Behavioural studies have shown that intracerebroventricular nociceptin may produce pain. In contrast, one study has shown that nociceptin selectively modulates spinal nociceptive events by reducing wind-up and post-discharge of neurones.1-3 The clinical significance of the peptide remains to be explored fully.

There is good evidence that opioid peptides are present in inflamed synovial tissue and can inhibit pain after knee surgery through an action specific to intra-articular opioid receptors.4 Nociceptin receptors have so far been identified predominantly in the CNS and a few peripheral organs such as the intestine, spleen and vas deferens.5 The association of these peripheral receptors with pain syndromes is of considerable interest, representing a new peripheral target for the management of pain. In this study, we determined whether NCR and its ligand nociceptin were located in human synovial tissue and fluid.

Methods and results

After obtaining approval from the Ethics Committee and written informed consent, we studied 21 patients undergoing total knee replacement. Initially, 10 ASA I–III patients, aged 62–83 yr, with a diagnosis of either osteoarthritis or rheumatoid arthritis were recruited for NCR and nociceptin identification. However, because of technical difficulties with the binding assay, it was necessary to recruit a second group of patients. Thus for receptor identification, 11 ASA I–III patients, aged 66–84 yr, with a diagnosis of osteoarthritis, were recruited. Patients with a BMI >30 kg m⁻² or who had previous intra-articular injection were excluded. Both tissue and fluid were removed by the surgeon at the start of surgery, immediately after skin incision and exposure of the knee joint.

Synovial tissue collection and [3H]nociceptin binding assay

A small amount of synovial tissue was mixed immediately with isopentone 0.5 ml. The samples were stored on ice.
Table 1 Lack of significant specific [3H]nociceptin binding to knee joint cartilage tissue. Data are from saturation analyses performed on tissues from each patient. For clarity, only binding at the saturating dose ([3H]nociceptin 16 nmol litre\(^{-1}\)) is shown. OA—diagnosis of osteoarthritis.

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before transfer to liquid nitrogen and subsequently batch processed. Tissue was cut into small pieces using dissecting scissors and then homogenized in ice-cold Tris HCl buffer (pH 7.4 at 4°C) using a Polytron (30 s), followed by ten up-down strokes in a glass Teflon homogenizer. The tissue preparation was then filtered through four layers of medical gauze to remove non-homogeneous matter. The resultant membrane filtrate was centrifuged twice for 10 min at 48 000 \(g\) (4°C), with an intermediate re-suspension of the first pellet in fresh buffer. Further pellets were re-homogenized in fresh Tris HCl to give a tissue concentration of at least 200 mg ml\(^{-1}\).

For the assay, 250 \(\mu l\) of tissue homogenates were added to [3H]nociceptin (increasing concentrations up to 16 nmol litre\(^{-1}\)) and buffer (containing 0.01% bovine serum albumin (BSA)) to give a final incubation volume of 500 \(\mu l\). Non-specific binding was measured in the presence of nociceptin 1 \(\mu mol \)litre\(^{-1}\). Incubations (25°C for 1 h) were terminated by filtration through G/F/B filters (pre-soaked in 1% polyethyleneimine) followed by 3 x 5 ml washes with ice-cold Tris HCl buffer. Filters were then counted for radioactivity using liquid scintillation counting.

**Synovial fluid sampling, nociceptin extraction and radioimmunoassay**

Synovial fluid aspirate was mixed immediately with an equal volume of trifluoroacetic acid (TFA:1% v:v) and frozen before batch extraction and assay. Acidified synovial fluid samples were loaded onto C-18 Sep-Pak cartridges and washed twice with 0.1% TFA. Nociceptin was eluted with 0.1% TFA 3 ml in 60% acetonitrile and then freeze dried. The reconstituted eluate was subjected to radioimmunoassay\(^6\) using a commercially available kit (Phoenix Pharmaceuticals Inc, CA, USA) with a minimum sensitivity of 0.5 pg ml\(^{-1}\).

There was no significant concentration-related or saturable [3H]nociceptin binding to any of the synovial tissue samples (Table 1). In addition, we failed to detect nociceptin in synovial fluid. In all patients, nociceptin concentrations were below the limit of detection of our assay (<0.5 pg ml\(^{-1}\)). We have reported previously cerebrospinal fluid and plasma nociceptin concentrations of 50–60 pg ml\(^{-1}\) and 7–13 pg ml\(^{-1}\), respectively.\(^6\)

**Comment**

We were unable to identify the nociceptin receptor or nociceptin in human synovial tissue. This receptor has been identified previously in mouse vas deferens, intestine and spleen. To our knowledge, there are no reports of identification of peripheral human NCR. However, we have detected nociceptin in human cerebrospinal fluid and plasma of parturients.\(^6\) If nociceptin is associated with pain, it might be reasonable to suggest that concentrations would be increased in pain states. In our study, patients with either osteoarthritis or rheumatoid arthritis and who were experiencing varying degrees of pain were used for identification of nociceptin. Despite this, concentrations of nociceptin were below the detection limit of our assay.

The exact role of nociceptin remains unclear with support for both pronociceptive and analgesic actions.\(^1–3\) Evidence to date suggests that nociceptin exerts distinct supraspinal and spinal actions. In behavioural studies, intracerebroventricular injection of nociceptin has been shown to antagonize the analgesic effects of stress and morphine. The mechanism of its antagonistic effects has not been elucidated fully but there is some evidence that nociceptin inhibits firing of neurones in the periaqueductal grey matter. In contrast to its supraspinal antianalgesic action, nociceptin produces analgesia when injected intrathecally, possibly by reducing wind-up and post-discharge of neurones.\(^2–3\)

Neither NCR nor nociceptin were identified in human synovial tissue or fluid and therefore intra-articular administration of nociceptin or nociceptin analogues does not represent a useful direct strategy for the management of joint-associated pain.

**References**