Sensory afferent properties of immobilised or inflamed rat knees during continuous passive movement

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We studied the sensory afferent properties of normal, immobilised and inflamed rat knees by recording the activity of the medial articular nerve (MAN).

When the knee was inflamed by kaolin-carrageenan or immobilised for six weeks, MAN activity significantly increased during rest and continuous passive motion (CPM). The maximal discharge rate tended to increase depending on the angular velocity of the CPM. When the knees were then rested for one hour before again starting CPM, activity was further increased at the initial CPM cycle, the ‘post-rest effect’. Analysis of the conduction velocity showed that 94% and 66% of spike units on the recorded discharge of the immobilised and inflamed knees, respectively, belonged to fine nerve fibres.

Our findings show that the sensory receptors in the knee are sensitised in a similar manner by immobilisation and by inflammation, suggesting a relationship to pain. The post-rest effect may be related to a characteristic symptom of osteoarthritis called ‘starting pain’.

Received 21 November 1997; Accepted after revision 8 January 1998

Immobilisation is the most common orthopaedic treatment for musculoskeletal injury, deformity, and inflammation. After prolonged immobilisation, however, complications such as joint contracture and atrophy of muscle and bone may occur. Experimental immobilisation used as a model for osteoarthritis has resulted in morphological degeneration and biochemical changes in the joint cartilage. Little is understood, however, about how sensory afferent information is modulated after immobilisation.

Schaible and Schmidt studied the physiology of joint receptors in cat knees which had been inflamed by the administration of kaolin and carrageenan. They reported increased ongoing activity and afferent discharge from nociceptive nerve endings in response to innocuous mechanical stimulation and passive movement. The mechanism underlying these phenomena is called ‘peripheral sensitisation’ in which high-threshold mechanoreceptors are recruited during normal joint movement.

In patients with osteoarthritis the most common complaints are resting pain and pain on initiating movement. This is often alleviated after a period of exercise. To understand the clinical characteristics of joint pain, we have studied the sensory properties of sensitised joints under controlled movement. Our aim in this study was to analyse quantitatively afferent fibres in the immobilised and inflamed rat knee during continuous passive movement (CPM).

Materials and Methods

Experimental procedure. We used 27 adult male Wistar rats weighing 250 to 350 g. They were anaesthetised with 2% to 4% halothane with controlled ventilation by tracheal intubation. The animals were then decerebrated at the level of the pons by suction ablation. One hour later, without anaesthesia, the skin on the medial side of the right thigh was incised from the inguinal fossa to a point immediately below the medial condyle of the tibia. The saphenous nerve and all of its branches except for the medial articular nerve (MAN) were severed leaving an appropriate length for recording. The periarticular subcutaneous tissue of the knee was simultaneously released from the medial patellar retinaculum. At the recording site, a paraffin oil pool was prepared using the surrounding skin and a pair of bipolar platinum recording electrodes attached to the saphenous nerve. Recording was confirmed by the observation of a discharge in response to mechanical probing of the medial and anteromedial aspects of the knee innervated by the MAN (Fig. 1).

The right hind foot was attached to a bar of a linear motor (model 2LM 1021-SA; Oriental Motor Co Ltd, Tokyo, Japan), which provided CPM and automatically moved the knee over a range of 10 to 100° with angular...
velocities of 9, 22.5 and 32°/s. After recording the activity during rest and CPM at each velocity the knee was arrested in flexion at 100° for one hour. The apparatus was then restarted to determine the effect of CPM on MAN activity. The final test was performed at an angular velocity of 32°/s.

To determine the types of fibre involved in MAN activity, the conduction time between two electrodes was measured by spike-averaging using a window discriminator. The conduction velocity of individual spike units was then calculated from the conduction time and the distance between the two electrodes.

**Experimental models.** The animals were divided into two groups and a control group of five normal knees.

The first group consisted of eight rats with kaolin-carrageenan-induced arthritis (KC group). Inflammation of the knee was produced in two steps according to the technique of Schaible and Schmidt. First, 30 µl of a 4% suspension of kaolin and distilled water was injected into the cavity of the joint. Secondly, passive mobilisation was performed for 15 minutes. Forty-five minutes later, 15 µl of a 2% carrageenan suspension was injected. Recording of activity in the MAN was begun three to six hours after the last injection.

In the second group of 14 animals (I group) the right hind limb was immobilised by deep flexion for six weeks using wire and an aluminium splint, similar to the method described by Wilson and Dahners. During this period, the animals were allowed free movement and access to food and water.

**Quantitative analysis.** MAN activity was quantified by counting the number of spikes plotted on the thermal printer (Thermal array recorder; Nihon Kohden, Tokyo, Japan) every 0.25 seconds. Resting activity was assessed from the data obtained 30 seconds before starting CPM. MAN activity during CPM was measured using the information from the fifth to the tenth movement after CPM was begun. MAN activities during rest and CPM are expressed as the mean and maximal values of the discharge rate. When CPM was restarted after one hour of arrest, the mean discharge rate of the first movement was determined for the next analysis (Fig. 2).

We used the Student’s t-test for comparison of data.

**Results**

**The effects of immobilisation and inflammation on MAN activity.** Figures 3 and 4 show MAN activity in normal, inflamed and immobilised knees during rest and CPM.

Activity was minimal in the five normal knees during rest and CPM. Slight, continuous activity at rest was seen in only one joint. CPM did not alter MAN activity even after one hour of arrest.

In the KC group MAN activity during rest significantly increased four to ten hours after the kaolin injection. Activity during CPM also increased in all animals at the same time. The discharge rate was maximal in four joints during the turning phase from flexion to extension and in five during that from extension to flexion. The rate was max-
imal in one joint during the extension phase (Fig. 5).

In the I group, MAN activity was continuous and increased during CPM in all the animals. In six knees the discharge rate was maximal during the turning phase from flexion to extension, in six from extension to flexion and in three during extension and flexion (Fig. 6).

The discharge rate in most inflamed and immobilised joints was maximal around the turning phase between flexion and extension. The discharge pattern recorded from each animal remained constant throughout the experiment.

The mean values for the discharge rate at rest were 1.02, 22.7 and 26.0 spikes/s in the normal, KC and I groups, respectively (Fig. 7). During CPM they were 1.54, 40.2 and 71.0 spikes/s (Fig. 8). The discharge rates of the KC and I groups during rest and CPM were significantly higher than those of the normal group.

**Effect of the angular velocity of CPM.** We analysed the relationship between the maximal discharge rate and the angular velocity of CPM. The mean values for the maximal discharge rate of the KC group during CPM were 46.2, 52.2 and 60.4 spikes/s at 9, 22.5 and 32°/s, respectively (Fig. 9). The difference in the discharge rate between 22.5 and 32°/s was statistically significant. Of the 14 joints of the I group, the mean values for the maximal discharge rate were 97.6, 112.8 and 125.2 spikes/s at 9, 22.5 and 32°/s, respectively (Fig. 10). In the I group, the increase in the discharge rate depending on the angular velocity was statistically significant. Under both conditions, the discharge...
MAN activity of each joint with kaolin-carrageenan-induced arthritis. The length of each bar indicates the degree of activity (low, moderate, high).

MAN activity of each immobilised knee. The length of each bar indicates the degree of activity (low, moderate, high).

Mean discharge rates during rest (N group = normal knees; KC group = kaolin-carrageenan-induced arthritis; I group = immobilised knees).

Mean discharge rates during CPM (N group = normal knees; KC group = kaolin-carrageenan-induced arthritis; I group = immobilised knees).
Effect of CPM arrest on MAN activity. In the KC and I groups MAN activity increased quickly during the initial cycle for CPM after arrest for one hour. The discharge rate stabilised after a few cycles of movement (Fig. 2). We called this the ‘post-rest effect’ and it was present in all animals in the KC and I groups.

The mean value of the discharge rate in the KC group was 57.4 spikes/s during the initial CPM cycle which then decreased to 40.2 spikes/s; the difference was statistically significant. In the I group the mean value was 110.9 spikes/s at the initial CPM cycle which then decreased to 71.0 spikes/s; this difference was also statistically significant (Fig. 11).

Impulse conduction velocities of spike units. In 17 identified spike units in the KC group the conduction velocities ranged from 5.88 to 25.0 m/s and 16 (94%) were below 20.0 m/s. In the I group they ranged from 3.31 to 33.3 m/s and 35 (66%) were less than 20.0 m/s.

Discussion

Medial articular nerve (MAN). The knee is a convenient site for analysing afferent fibres because of its ease of handling and well-defined innervation. The major nerves innervating the knee are the medial and posterior articular nerves. Receptors for the MAN are located at the medial aspect of the joint capsule, the medial collateral ligament and the patellar tendon. Selective MAN activity can easily be recorded from the saphenous nerve by transecting the other branches. Afferent recordings are not disturbed by CPM.

Afferent properties of normal knees. Our findings showed that there is very little afferent activity in normal rat knees at rest or during CPM. Schaible and Schmidt found slight activity during passive movement in the cat. Several reasons can account for this difference. First, the rat knee is much smaller than that of the cat and therefore may have fewer nerve endings. Secondly, the amount of stress on the periarticular tissue caused by the range of movement (flexion of the knee from 10 to 100°) is probably quite small. Because the range of movement is not associated with weight-bearing, activities at rest and during CPM in inflamed or immobilised knees indicate that there is direct joint sensitisation.

Effects of inflammation and immobilisation on MAN activity. We found a significant increase in MAN activity during rest in both inflamed and immobilised knees. Schaible and Schmidt recorded group-III and group-IV joint afferent activity from the cat MAN. They reported that the resting activity of group-III afferents in the inflamed knee was irregular and highly frequent (mean 1.0 spike/s; range 0.1 to 2.0 spikes/s). The discharge rates which we obtained from the inflamed rat knees (mean 22.7 spikes/s) were considerably higher than those of Schaible and Schmidt, although our recording contained a larger number of nerve fibres.
extension. They did not measure MAN activity within the continuous movement phase and its relationship to movement velocity.

Our study has shown that the discharge rates within a movement phase varied among animals with kaolin-carrageenan-induced arthritis and with immobilised knees, although activity was highest in most during the turning phase between extension and flexion. The difference in the time of the maximal discharge rate during the movement phase seemed to be associated with the location of the sensitised nerve terminals. The restricted full flexion with terminal pain that is generally seen in patients with arthritis of the knee may be related to this phenomenon.

We have also shown that MAN activity in sensitised knees tended to increase with increasing angular velocity. This is thought to reflect a general characteristic of mechanoreceptors, namely a velocity-dependent increase in response.

**Joint afferents and types of joint sensation.** There are four types of sensory nerve ending in joints. Dense nerve terminals are located in the fibrous layers of capsules, menisci, ligaments and around small vessels. Type-I and Ruffini endings are structurally similar and basically sensitive to mechanical stretching. Type-II endings are like Pacini corpuscles and are sensitive to high-frequency vibration and high-velocity changes in joint position. Type-III endings resemble the Golgi tendon apparatus and are mostly distributed among extrinsic and intrinsic ligament tissues. This type of ending adapts slowly and has a high mechanical threshold. Type-IV endings are free nerve endings and involve those sensitive to noxious mechanical or biochemical stimuli. Known stimulants of nociceptive nerve endings include mechanical stimuli and chemicals such as bradykinin, prostaglandins, serotonin and neuropeptides. Schäible and Schmidt identified an increased response in type-IV receptors in cat knees inflamed by kaolin and carrageenan. This may be accompanied by joint pain; Sluka, Willis and Westlund observed behavioural changes in animals with inflamed joints.

Generally, afferent discharge from nociceptors is conducted along group-III or group-IV sensory fibres that have a conduction velocity below 20.0 m/s. Our study has shown that the conduction velocity of most afferent discharges in inflamed and immobilised knees was below 20 m/s. Therefore, most of the activities observed in these two models are probably from nociceptive afferent fibres and are manifested as joint pain.

**Post-rest effect.** We observed a ‘post-rest effect’ which is a further increase in MAN activity occurring during the first few CPM cycles after a period of rest. It was seen in all the inflamed and immobilised knees after at least 10 to 30 minutes of rest. This phenomenon appears to be an important characteristic of sensitised joint receptors because it may be related to ‘starting pain’, which is a common symptom shown by patients with arthritis of the knee.

**Effects of immobilisation.** Stress deprivation alters the morphological, biochemical, and biomechanical characteristics of various components of synovial joints. Immobilisation has been used as a model for arthritis. The effect of immobilisation on the sensory afferent properties of joints, however, has not been examined. We have studied the effect of immobilisation on afferent sensitisation in joints and have compared the afferent properties of immobilised knees with those of kaolin-carrageenan-induced arthritis.

We found that the nerve endings were sensitised in a similar manner by both immobilisation and by inflammation. Other investigators have shown increase in the intraarticular effusion, hydrostatic pressure and local temperature in immobilised joints. With an extended period of immobilisation, the simultaneous turnover of proteoglycans in various tissues, especially in the capsule and in cartilage, finally leads to a state resembling that of the osteoarthritis seen in man. Evans et al. showed that after immobilisation for 30 days adhesions developed between fibrofatty connective tissue and underlying cartilage surfaces. Where two cartilage surfaces converged there was marked atrophy and thinning. After 60 days, ulceration of the...
cartilage was found in areas of compression, indicating that immobilisation produces joint degeneration.

Joint swelling and physiological changes in the afferent properties show that immobilisation induces a chronic arthritic state after a relatively short period. During the six weeks of immobilisation in our study, chronic inflammation and structural changes in the capsule and ligaments were induced in the joints, which finally sensitised nociceptive terminals and mechanoreceptors.

**CPM as a clinical tool.** Continuous passive motion is widely applied to patients who have had joint surgery in order to help the joint to recover mobility and to maintain cartilage after operation the joint may be exercised passively with relatively little pain, probably because the post-rest effect does not occur during CPM. A low angular velocity should be applied to avoid increasing the instantaneous discharge rate. We found that high resting MAN activities decreased after a period of passive movement. These observations clarify the beneficial effect of CPM.

We wish to thank Professor T. Matsuno for encouragement, A. Tokuhiro, MD, and M. Watakabe, PhD, for help with the design of this study and Miss Y. Masuya for technical assistance.

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