THE NORADRENERGIC INNERVATION OF RAT KNEE JOINT ARTICULAR CAPSULE AND LIGAMENTS

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Noradrenaline levels and distribution in different components of rat knee joint were studied using radioenzymatic assay and glyoxylic acid histofluorescence techniques.

The articular capsule possessed the highest noradrenaline content, and the levels of noradrenaline in cruciate ligaments were slightly higher than in collateral ligaments. In all joint components, noradrenergic nerve fibres were associated almost exclusively with the blood vessels.

The possible involvement of a noradrenergic action on joint microcirculation in the pathophysiology of rheumatoid arthritis is discussed.

A detailed knowledge of the innervation of joints is of particular interest since neural mechanisms appear to be implicated in the pathophysiology of rheumatoid arthritis (8, 11). Moreover it has been demonstrated that the release of the putative peptide neurotransmitter substance P from peripheral nociceptive terminals and the release of catecholamines from postganglionic sympathetic neurons produce, in joints, the physiological changes commonly associated with acute inflammation (12).

The available anatomical findings are limited to studies carried out using classic neurohistological or electron microscope techniques and aimed, almost exclusively, at the identification of afferent receptor structures within the different anatomical components of joints (2, 3, 7, 9). No detailed data have been so far published, to our knowledge, concerning the efferent innervation of joints.

However, efferent pathways may be involved in joint inflammation. For instance, hyperactivity of the sympathetic system causes flares of rheumatoid arthritis, and sympathectomy reduces the severity of joint injury in experimental adjuvant-induced arthritis (12).

In view of this, we have analyzed the noradrenergic innervation of rat knee joint capsule and ligaments, using neurochemical and neurohistochemical techniques. The rat was chosen for study because of the very similar anatomical organization of its knee joint to that in humans (14).
MATERIALS AND METHODS

Thirty-eight male Wistar rats aged 3 months were used. Thirty animals were used for neurochemical assays; the remaining 8 rats were used for neurohistochemistry. Animals were anaesthetized with diethyl ether, the knee joint was exposed using a stereomicroscope, and the articular capsule, the anterior and posterior cruciate ligaments, and the tibial and fibular collateral ligaments were removed for neurochemical assays.

Corresponding tissues from the right and the left side of 3 rats were pooled in order to obtain material with noradrenaline levels suitable for neurochemical determination. Tissues were washed in ice-cold saline, blotted, weighed and homogenized in 400 mM perchloric acid at 0°C using a glass-to-glass homogenizer. Ligaments were first disrupted with a polytron homogenizer for 1 min. In the case of the articular capsules, polytron treatment was not necessary. Homogenates were centrifuged for 10 min at 4°C at a speed of 3,000 g/min. The supernatants were removed for noradrenaline assay and the pellets were further homogenized in 1 M NaOH and diluted in distilled water to give a tissue concentration equivalent to 1 mg of tissue wet weight/ml distilled water. The diluted alkaline extracts were then taken for protein measurement according to Lowry et al. (13), using a standard of bovine serum albumin.

The tissue noradrenaline content was measured by the radioenzymatic method of Da Prada and Zurcher (6) using a commercially available kit (Amersham, U.K.). For neurohistochemistry, tissues were dissected as indicated above and immersed for 90-120 min at room temperature in a 2% glyoxylic acid solution made up in 0.1 M phosphate buffer (final pH 7.2). At the end of incubation, each ligament was divided into 2 halves.

The specimens were air dried at room temperature, heated at 100°C for 4 min, stretched flat on microscope slides, mounted in liquid paraffin and observed using a Zeiss II photomicroscope equipped with epi-illumination. Further details on the procedure followed have been reported in an earlier paper (1).

The number of perivascular fluorescent nerve fibres and varicosities within the whole mounts were counted directly in the microscope using a X 40/0.95 objective and a X 10 ocular, and the length and diameters of blood vessel segments were measured using a calibrated eyepiece graticule.

RESULTS

The concentrations of noradrenaline in the articular capsule and in cruciate and collateral ligaments are shown in Table 1. Noradrenaline levels in the articular capsule were higher than those in the ligaments, and the noradrenaline content of the cruciate ligaments was slightly higher than that in the collateral ligaments (table 1).

After exposure to glycoxylic acid, whole mounts of articular capsule and ligaments displayed a plexus of varicose perivascular nerve fibres located primarily around arteries and arterioles (Figs. 1, 2). In contrast, the veins and venules were sparsely supplied with nerve fibres (Fig. 3). No fluorescent nerve fibres associated with nonvascular structures were observed in either articular capsule or ligaments (Fig. 2).
TABLE 1. Noradrenaline content of rat knee joint articular capsule and ligaments

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>NORADRENALINE (µg/gr. tissue)</th>
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<tbody>
<tr>
<td>ARTICULAR CAPSULE</td>
<td>10</td>
<td>0.18±0.04a</td>
</tr>
<tr>
<td>ANTERIOR CRUCIATE LIGAMENT</td>
<td>10</td>
<td>0.10±0.01</td>
</tr>
<tr>
<td>POSTERIOR CRUCIATE LIGAMENT</td>
<td>10</td>
<td>0.09±0.02</td>
</tr>
<tr>
<td>TIBIAL COLLATERAL LIGAMENT</td>
<td>10</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>FIBULAR COLLATERAL LIGAMENT</td>
<td>10</td>
<td>0.06±0.01</td>
</tr>
</tbody>
</table>

Results are the mean±S.E.M. of determinations per pool of animals (see text) carried out in duplicate; "n" is the number of different pools of animals examined.

a P<0.001 vs. cruciate or collateral ligaments (two-tailed Student's "t" test).

Fig. 1. Rat knee joint. Articular capsule. Glyoxylic acid histofluorescence. Fluorescent varicose nerve fibres are organized in a periarterial plexus. The density of nerve fibres and varicosities is higher in arteriolar branches than in the parent artery (A). × 110

Fig. 2. Rat knee joint. Tibial collateral ligament. Glyoxylic acid histofluorescence. The plexus of perivascular noradrenergic fibres is denser at arteriolar (a) than at arterial (A) level. × 280

Fig. 3. Rat knee joint. Anterior cruciate ligament. Glyoxylic acid histofluorescence. The picture shows a vein supplied with a sparse plexus of noradrenergic nerve fibres. × 280
As summarized in Table 2, the density of innervation was found to be higher in arterioles than in arteries. However, no apparent differences were seen in the density or pattern of blood vessels innervation between the articular capsule and the ligaments. Thus, tissue noradrenaline contents of the various joint components probably reflect different numbers of blood vessels present within these structures, rather than different densities of innervation.

**DISCUSSION**

In recent years, evidence has accumulated suggesting that the nervous system may play an important role in the pathogenesis of rheumatoid arthritis (11, 12). Although sensory mechanisms and primarily neuropeptide systems seem to have major influences (4, 8, 10-12), a possible contribution of the sympathetic nervous system has also been suggested (11, 12). This hypothesis was based on the observation that local application of noradrenaline in patients with causalgia produces hyperalgesia (16), and that sympathectomy significantly attenuates adjuvant-induced arthritis in the rat (10, 12).

Our findings show that in the rat knee joint the noradrenergic sympathetic nerve supply is limited to arteries and arterioles, with the arterioles being the most densely innervated. Thus, if endogenous noradrenaline is implicated in genesis of rheumatoid
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arthritis, this effect is probably mediated by an action on joint microcirculation. However, there is also a possibility that the release of noradrenaline from perivascular varicosities, which correspond to sites of noradrenaline release (5), may indirectly modulate the activity of immunocompetent cells of synovial tissue or the function of primary afferent nociceptors (see 11, 12).

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REFERENCES