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Mechanoreceptors in collateral knee ligaments: an animal experiment

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Abstract The mechanoreceptors in the collateral ligaments of the knee joint in rat hindlimbs were studied. In group II ($n=10$) the femoral and obturator nerves were sectioned. In both groups III and V ($n=20$) the sciatic nerve was sectioned. In group V ($n=10$) the sectioned sciatic nerve was sutured 4 weeks after sectioning. In group IV ($n=10$) all three nerves were sectioned. Group I ($n=10$) served as control. After 4 months all animals were killed. The ligaments of the knee joint were preserved and stained with gold chloride, paraffin-embedded and cut in sagittal serial sections. The results showed that 4 months after partial or total denervation of the limb, there was necrosis and a decrease in the number of mechanoreceptors, which was dependent upon the severity and site of the lesion. After suture of the sciatic nerve the increase in mechanoreceptors suggested a regenerative process.

Résumé La présente étude avait pour but d'analyser chez le rat les mécanorécepteurs des ligaments collatéraux de l'articulation du genou dans les membres postérieurs normaux, après dénervation, et après suture du nerf sectionné. Pour ce faire, nous avons étudié le nombre de mécanorécepteurs par unité de volume. Cinq groupes distincts ont été analysés : Groupe I (groupe témoin) : aucune intervention n'a été effectuée. Groupe II : exposition et résection d'un segment du nerf fémoral et du nerf obturateur. Groupe III : exposition et résection d'un segment du nerf sciatique. Groupe IV : des secteurs des trois nerfs ont été exposés et extraits. Groupe V : quatre semaines après résection du nerf sciatique, ce dernier a été suturé. Au bout de quatre mois, les ligaments ont été colorés en bloc en utilisant la technique du chlorure d'or puis inclus dans la paraffine,

et des séries de coupes sagittales ont été examinées. Les résultats suggèrent que, quatre mois après dénervation, il y a nécrose et diminution du nombre des mécanorécepteurs et que ce processus augmente selon l'intensité et la localisation de la lésion. L'élévation du nombre de ces récepteurs après suture du nerf sciatique suggère l'existence d'un processus de régénération des mécanorécepteurs.

Introduction

Mechanoreceptors work as transducers, converting physical energy expressed as tension into nervous signals [7, 34]. They play an important role in the control of movement, and provide information to the central nervous system when a joint is injured [32]. The morphology and distribution of mechanoreceptors in the joint capsule been described in humans [8, 11, 17, 19, 21, 22, 27, 34], and in animals [2, 4, 13, 26, 28]. The presence of mechanoreceptors after ligament transection [12], in autologous tendon grafts [5], and in regenerated menisci [14] have also been described. Zelena and Zarachova [31] have shown reinnervation of the Pacini corpuscles after sciatic nerve damage in rats.

In order to shed light on the behaviour of mechanoreceptors in denervated ligaments, we analysed the number of receptors in the collateral ligaments of the knee joints of rats. Normal and denervated hindlimbs were examined as well as partial denervated limbs after repair of the injured nerve.

Material and methods

Fifty adult female Wistar rats, each weighing 250 g, were used. All the animals were kept in standard plastic cages, with light (12 h on 12 h off), temperature (23 ± 10 °C) and humidity (60%) conditions unmodified throughout the study. European Union guidelines for the care of animals used for experimentation and other scientific purposes (Guideline 86/609/EEC and R.D. 223/88 B.O.E.) were followed. Five groups of rats were considered: group 1 ($n=10$), a non-treated control group where no surgical intervention was performed. The remaining 40 animals were anaesthetized

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with a mixture of 50 mg ketamine, 20 mg diazepam, and 1 mg atropine dissolved in 4 ml of bidistilled water, at a dose of 1 ml/100 g body weight, and distributed into four groups. In group II ($n=10$), the femoral and obturator nerves were exposed under sterile conditions. Both nerves were sectioned and 1 cm was removed from each. In group III ($n=10$) and V ($n=10$) the sciatic nerve was exposed, sectioned and 2 cm was removed. The remaining nerve ends were inverted and buried in muscle. In group V the sciatic nerve was repaired 4 weeks after sectioning. In group IV ($n=10$), all three nerves of the left hindlimb were sectioned.

After 4 months all rats were killed. The medial and lateral collateral ligaments of the left knee joint including the capsular periligamentary connective tissue, were preserved, dissected and placed in ice-cold 1% NaCl. The ligaments were stained in bulk with the gold chloride technique [15] as follows: each ligament was placed in a small glass vial and covered by a solution of fresh lemon juice mixed with 88% formic acid solution (3:1, v/v). The vials were then kept in darkness for 10 min. The solution was then replaced by a 1% aqueous solution of gold chloride for another 10 min, and also kept in the dark. Subsequently, ligaments were immersed in 25% formic acid, and kept in the dark for 10 h at 40 °C. The ligaments were finally rinsed three times with 70% ETOH for 10 min before being placed in glycerol for 24 h. The tissue was then dehydrated and paraffin-embedded, and 25 μ m thick sagittal serial sections were cut and mounted for light microscopic examination.

The classification of Freeman and Wyke [7] and the detailed histological study reported by Zimny et al. [33, 34] were followed. Four types of mechanoreceptors could be distinguished: type I or Ruffini-like end organs, which are thinly encapsulated globular corpuscles; type II or Pacini-like endings, thickly encapsulated conical corpuscles; type III or Golgi-like thinly encapsulated fusiform corpuscles and type IV, i.e. free nerve endings.

In 25- μ m serial sections, the type and number of mechanoreceptors within ligament and capsular periligamentary connective-tissue were recorded. The same receptors were identified several sections. The number of receptors per mm³ tissue was calculated using a Videoplan-Kontron image analyser.

Results

Group I. Control

Ruffini receptors (type I) represented 9.72% (Fig. 1a) and Pacini corpuscles 11.43% of the total number of receptors per ligament (Fig. 1b). Both receptor types were found in the periligamentary tissue. Golgi corpuscles (type III) represented 4.36% and free nerve endings (type IV) constituted 74.46% of the receptors per ligament (Fig. 1c). Both types were observed in the periligamentary tissue as well as in the ligament itself.

Group II. Injury of femoral and obturator nerves

Muscular atrophy in the anterior part of the thigh and cellular necrosis in the ligament were observed. In 6 out of 9 of the lateral ligaments neither Pacini nor Golgi mechanoreceptors were observed (Table 1). Necrotic receptors were observed (Fig. 2).

Group III. Injury of the sciatic nerve

Muscular atrophy in the thigh and the leg was observed. In the foot there was skin atrophy and ulceration around

Fig. 1a,b Histological appearance of the mechanoreceptors of a rat knee joint. **a** Ruffini receptor. An axon enters the corpuscle. A thinner capsule is observed. **b** Pacini corpuscle. It is an oval structure. In the center of the receptor a hyperchromatic lineal structure is seen, surrounded by lamellar structures. **c** Free nerve ending. Gold chloride stain, $\times 400$

the heel. Cellular necrosis in the ligaments was seen. In the lateral ligaments only free nerve endings were seen. In the medial ligaments no Ruffini no Golgi receptors were seen in 7 out of 9 ligaments (Table 1).

Group IV. Injury of the femoral, obturator and sciatic nerves

Marked atrophy and deformity were observed. The capsular and ligamentous tissues were atrophied. Achromatic and cellular necrosis was seen. Only necrotic receptors were observed (Fig. 2).

Group V. Suture of the sciatic nerve

Mild muscular atrophy and a zone of subcutaneous skin adherence was seen in the leg. Mechanoreceptors were

Table 1 Number of mechanoreceptors per mm³ (mean±SD) in collateral ligaments (CL) of knee joints studied

	Control		Injury femoral and obturator nerves		Injury sciatic nerve		Suture sciatic nerve	
	Lateral CL <i>n</i> =10	Medial CL <i>n</i> =10	Lateral CL <i>n</i> =9	Medial CL <i>n</i> =8	Lateral CL <i>n</i> =10	Medial CL <i>n</i> =9	Lateral CL <i>n</i> =9	Medial CL <i>n</i> =9
Type I Ruffini	1.08±0.62	0.57±0.13	0.06±0.03 ^d	0.18±0.05 ^d	0	0.07±0.14 ^{a,d}	0.19±0.02 ^e	0.12±0.05 ^e
Type II Pacini	0.97±0.21	0.97±0.28	0.07±0.04 ^{b,d}	0.26±0.08 ^d	0	0.11±0.04 ^d	0.22±0.17 ^e	0.21±0.15 ^e
Type III Golgi	0.43±0.17	0.31±0.14	0.13±0.03 ^{c,d}	0.13±0.07 ^d	0	0.03±0.07 ^{a,d}	0.13±0.03 ^e	0.12±0.07 ^e
Type IV free nerve endings	6.96±1.22	5.67±1.01	1.06±0.19 ^d	0.93±0.25 ^d	0.63±0.13 ^d	1.18±0.23 ^d	2.21±0.13 ^e	2.32±0.43 ^e

^a Two cases with receptors, the remaining ligaments were empty.

^b Three cases with receptors, the remaining ligaments were empty.

^c Five cases were empty

^d $P<0.05$, compared with the control group; ^e $P<0.05$, compared with the injury sciatic group. Mann-Whitney U-test

Fig. 2a,b Histological appearance of necrotic mechanoreceptors of a rat knee joint. **a** Necrotic Ruffini receptor. **b** Necrotic Golgi receptor. Gold chloride stain, ×400

seen in both ligaments. In the lateral ligament the number of mechanoreceptors was greater than in group III. In the medial ligament an increase of free nerve endings was observed compared to group III (Table 1).

Discussion

In the present study it has been shown that the capsule-synovial complex and the collateral ligaments of the rat contain Ruffini endings, Pacini corpuscles, Golgi organ-like endings, and free nerve endings, as previously described in other animals [13,18]. After denervation there are histological changes that increase gradually from the group with a lesion of the femoral and obturator nerves to the group with a lesion of all three nerves. We have interpreted this as a mixture of neural and vascular injury [20]. The decrease in the number of mechanoreceptors with respect to the injured nerve seems to show a territorial distribution; the lateral ligament being the most affected.

Ruffini (type I) and Pacini (type II) receptors have been observed by us only in the periligamentary capsule synovial tissue, attached to the ligament. However, O'Connor and Gonzales [15], observed the presence of type I endings in the medial ligament of the cat knee joint. Ruffini receptors are one of the most sensitive mechanoreceptors to denervation. Jimmy et al. [32] define type I as low-threshold, slowly adapting receptors that respond to mechanical stress; and type II as dynamic, rapidly adapting mechanoreceptors. Type III or Golgi endings, are less numerous. Jimmy et al. [32] suggested that these receptors would become active only when joints were at the extreme of their range of movement. Type IV or free nerve endings are the least reduced in number after denervation.

It is difficult to compare the results of different studies. Petersen and Stener [16] suggested that there may be considerable variations in the structure of joint capsule receptors from species to species, joint to joint and even capsule to capsule.

When analysing the sciatic nerve suture group we observed an increase in the number of mechanoreceptors in the capsule-ligamentary structure, although they do not reach the level of the control group. This suggests the existence of a “mechanoreceptor regeneration process”.

Receptor regeneration is a well-known phenomenon [1, 9, 25]. Jirmanová et al. [10] have shown that the receptors regenerate after denervation, although they do so in smaller amounts and vary in size and structure.

It is known that mechanoreceptors are associated with two important structures: Schwann cells, from which corpuscle lamellar cells derive [23, 24, 29, 30] and the basal lamina that covers the cellular components of corpuscles [10].

Adler et al. [1] suggested a hypothesis for the mechanism of receptor replacement, which they called “supporting-cell conversion”. After receptor injury, these authors described the presence of unusual cells near the mechanoreceptors with the ability to transdifferentiate on receptors. According to Beresford [3], transdifferentiation means a switch in the phenotype of a cell that has acquired a stable working character. Growth factors such as

fibroblast growth factor and transforming growth factor could be essential in regulating the process of transdifferentiation, presumably through cooperative functions with components involved in the extracellular matrix [6].

Partial denervation of the posterior limb leads to a loss of mechanoreceptors in the collateral knee ligaments in mammals. In human clinical practice, the possibility of mechanoreceptor regeneration observed after nerve suturing suggests that the proprioceptive reflexes may recover after nerve injury, or ligament grafting.

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