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Quantification of mechanoreceptors in the canine anterior cruciate ligament

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Abstract Ten canine anterior cruciate ligaments (ACLs) were harvested while preserving their bony attachments. Specimens were stained using a modified gold chloride technique, divided into thirds, and serially sliced at 0.5 microns. The slides were viewed to count the mechanoreceptors present. The average numbers of receptors found were: proximal 67, middle 43, and distal 18 (ANOVA: $P<0.001$). The statistical test (Sheffé) revealed that the proximal third contained a greater mean number of receptors ($S=3.8$). No significant difference was found between the number of receptors in the middle and distal thirds ($S=0.85$).

Résumé Les ligament croisés antérieurs de dix chiens ont été prélevés avec leurs insertions osseuses. Un fixateur externe a été utilisé pour garder le ligament à sa longueur de repos. Les ligaments ont été colorés au chlorure d'or puis divisés en tiers proximal, moyen et distal. Chaque section a ensuite été coupée de façon sériée à intervalle de 5 microns et montée sur lame pour étude au microscope. Le nombre moyen de récepteurs par tiers du ligament étais: proximal 67, moyen 43 et distal 18. Les résultats de l'analyse de variance ont démontrés une différence entre les moyennes de récepteurs de chaque tiers ($P<0.001$). Le test statistique de Sheffé a montré que le tiers proximal contenait un plus grand nombre de récepteurs ($S=3.8$) alors qu'il n'y avait pas de différence significative entre les tiers moyens et distaux du ligament.

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Introduction

One of the most important stabilizing structures in the knee is the anterior cruciate ligament (ACL). The stabilizing function of this ligament is well known but its role in proprioception of the knee is unclear. Mechanoreceptors have been described in human as well as animal ACLs. Despite this presence the role these receptors play in proprioception is unknown.

The knee ligaments provide the knee with static stability. These ligaments prevent the knee from moving out of its normal plane of motion. Injury to these structures can result in instability and "giving out" of the knee. In the dog an ACL tear usually results in rapidly progressive osteoarthritis of the knee joint [1,4,5,7,12,13,18,19,20,23], but this progression is not necessarily true in humans [2,6,8,14,15,21,22].

Proprioception is the ability to perceive the position and motion of a joint in space. The mechanism by which the brain performs this task is not completely elucidated. Although some work has been done in this area, how much information is transmitted from the ligament mechanoreceptors to the brain is unknown. Furthermore, we still have no knowledge of the microscopic anatomy of the mechanoreceptors in the ligaments, and the amount of proprioception that these ligaments provide is a matter of debate. We feel that the first step towards understanding the role of mechanoreceptors in any joint is to delineate their distribution in these ligaments.

The purpose of this study was to attempt to identify the different types of mechanoreceptors in the canine ACL and then attempt to quantify these receptors. We wanted to be able to see where the receptors are located and how many are present. We also wanted to identify the region of the ACL that had the highest concentration of receptors to help us understand the proprioceptive role of this ligament.

Materials and methods

Inclusion criteria

ACL specimens were obtained from canine knees immediately after the dogs were killed. To be included in this project the dog had to have been in good overall health and not have a known injury to its knee. Dogs that had been used in musculoskeletal research projects could not be involved in a project that traumatized the knee joint, and the leg or knee of the animal had to be frozen within 12 h of its being killed. The size of the dogs was not important. The specimens came from several different breeds but also included mongrels. If the dog was a part of another study, then its weight-bearing status was recorded.

Dogs with evidence of prior injury to their ACL were excluded. Furthermore, any animal that had evidence of a prior injury to any other knee ligament was excluded. The inability to preserve an adequate bone block from the origin or insertion of the ligament was grounds for exclusion of the ligament from the project. The presence of osteoarthritis in the knee meant exclusion of the knee. The fact that an animal was non-weight-bearing on that limb prior to harvest was not considered to be an exclusion criterion. Despite these strict criteria, no specimens needed to be excluded.

Specimen harvesting and preparation

Knee joints of 10 dogs were obtained after killing the animals. Most of these animals were killed as part of another research project. The weight-bearing status that each animal had on each leg was recorded. The dogs were all healthy. The knees were harvested within 2 h of killing and the complete knee joint resected. The knees were frozen at –30˚C until the author could dissect out the ACL from each knee.

The knees were opened using a medial parapatellar incision. The joint was inspected as were the ligaments and menisci. All muscle and fat attached to the tibia and femur was sharply dissected using a number 15 blade. The patella and its tendon were then removed from the tibial tubercle. The following structures were then removed from each knee: ACL, posterior cruciate ligament (PCL), and menisci. The bony insertion of the ACL and PCL were cut from the tibial plateau and femoral notch. Each animal’s PCL and menisci were examined and harvested for other unrelated studies using a similar protocol. The ligaments were then immediately refrozen at –30°C until further processing.

The ACL with its intact bony origin and insertion were then thawed out over night. The ligaments were held at their resting length using a special external fixator, specifically designed for this project. The ligaments were stained using a modified gold chloride stain as described by Zimny et al. [27].

The ligaments were sectioned in thirds so that there was a proximal, middle, and distal third for each ACL. From these ligaments at least three sections were selected for final processing. Each section was serially sliced at 5-μm intervals, using a microtome. The sections were then fixed to slides and covered with cover slips.

One author examined each slide and the mechanoreceptors present were counted and identified. The quantity of each type of receptor was noted. These data were written on data sheets and entered into a spreadsheet later. The slides were first visualized under low power and then at higher powers to identify each receptor. The position of each receptor was memorized, using specific landmarks on the slide (ligament architecture) to prevent accidentally counting the same mechanoreceptor twice. It occasionally took the examiner several different slices through the receptor to identify the receptor and/or distinguish it from artifact.

Statistical analysis

The total number of mechanoreceptors of each slide was calculated for each third of the ligament. The average number of mechanoreceptors was calculated by taking the sum of mechanoreceptors viewed in each section and dividing it by the number of slides in that section. An analysis of variance (ANOVA) was performed on these results. Alpha was set at 0.05 and Beta set at 0.80. *P* values less than 0.05 were considered significant. Significant values for the ANOVA were further investigated using the Scheffé A Posteriori test to determine where the difference was located.

Results

Two thousand slides were viewed, and two types of receptors were found. The majority of these receptors were Pacini receptors (Fig. 1). They made up the majority of all the receptors that were seen in any third of the ligament. Ruffini receptors were the second most frequent type of receptor found in the ACL (Fig. 2). Free nerve endings were seen but not felt to represent a mechanoreceptor since their function seems to be pain perception and autonomic nervous system regulation of blood flow.

Slide quality varied. Most slides were very clear and with little artifact. Some sections did show folding of the specimen edges. The thin serial slices helped the identification process, because they allowed the author to examine the section above and below to confirm the presence of a receptor.

In the proximal third of the ligament 323 Pacini receptors were noted. Only 100 were noted in the middle third and only 33 seen in the distal third. Fewer Ruffini receptors were found. Seventy were noted in the proximal portion, which represented the largest number of these receptors in a section. Only 7 receptors were seen in the distal section and 23 in the middle section.

The mean number of receptors seen per slide (Table 1) varied from 0.65 for Pacini receptors in the proximal third to 0.05 Ruffini receptors identified in the distal third. Because of the thinness of the slices the same receptors had to be viewed for at least 3 consecutive slides, before it was counted. The mean number of receptors per slide for the remaining thirds of the ligaments is shown in detail in Table 2.

The mean number of receptors per section also showed a predominance of Pacini receptors in the proximal third of the ligament. As we move distally in the ca-

Table 1 Mean number of receptors per slide in each third of the ligament

Mean receptors/slide	Pacini	Ruffini	Total
Proximal third	0.65	0.13	0.78
Middle third	0.31	0.07	0.38
Distal third	0.23	0.05	0.28

Table 2 The mean number of receptors in each segment of the ligament

Mean receptors/section	Pacini	Ruffini	Total
Proximal third	48	10	58
Middle third	25	5.8	30.8
Distal third	11	2.3	13.3

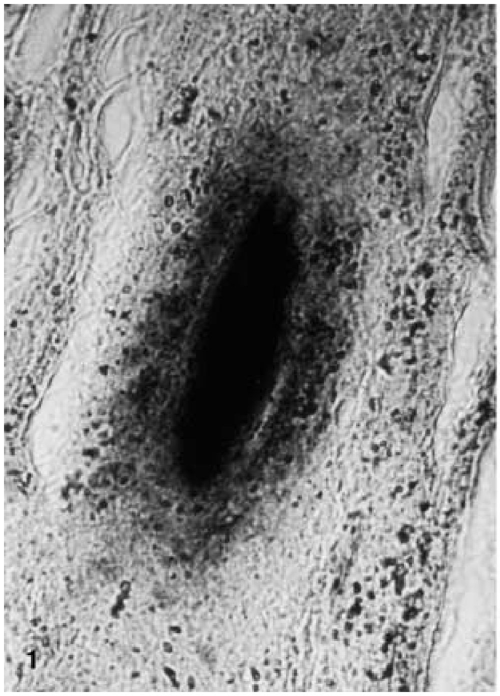

Fig. 1 Pacini receptor of canine ACL

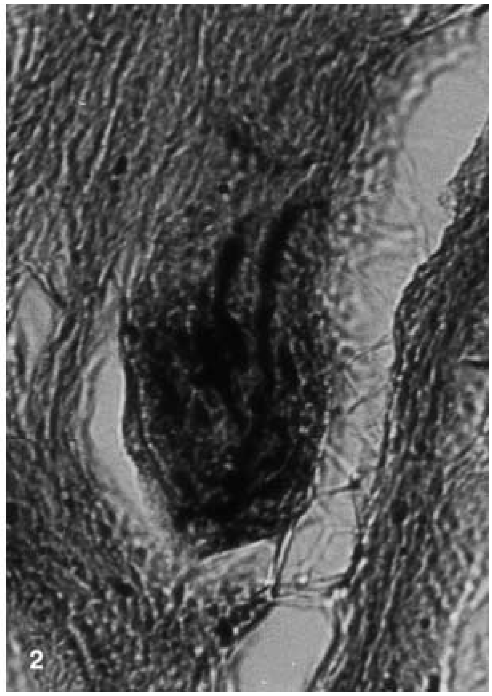

Fig. 2 Ruffini receptor of canine ACL

nine ACL, fewer receptors are visualized. This is true not only for the type of receptors but also in the sheer number of receptors seen.

Results of the ANOVA performed on the mean number of mechanoreceptors in each third of the ligament showed that there was a significant difference between the three means ($P<0.001$). The Sheffé A Posteriori test was then applied to the results to determine where this difference lay. The results showed a significant difference between the number of mechanoreceptors found in the proximal portion of the canine ACL and the number of receptors found in the middle and distal thirds ($S=3.8$). There was no significant difference found between the number of receptors in the middle and distal thirds ($S=0.85$).

Discussion

Mechanoreceptors are pressure-sensitive corpuscles that have been identified in the ligaments of almost all the joints of both humans and animals [3,9,10,11,16,17, 24,25,26]. These receptors have been shown to send impulses to the brain when the knee joint is flexed and extended as well as internally and externally rotated [11].

Despite the identification of all these mechanoreceptors, no studies have ever attempted to quantitate the number of receptors present in the ligaments. This basic fact has been overlooked. This study was an attempt to try to fill this void.

The canine knee is a good specimen on which to identify mechanoreceptors. Researchers have previously identified mechanoreceptors in the ACL of dogs. Furthermore, these researchers used gold chloride to identify them. Canine knees are easier to obtain than human cadaveric knees. The smaller size of the knees makes it feasible to take serial sections of the ACL. The smaller size of the ACL is also helpful when attempting to stain these ligaments. Smaller ligaments tend to have a more uniform dispersion of stain and are less likely to have artifacts. This is important when trying to count and identify mechanoreceptors on a slide.

The canine joint has some similarities and differences with the human knee. The same ligaments are found in each and their positions in the knee are similar. Both joints are hinge joints, but while the human knee joint can fully extend, the dog is unable to perform this function. The ACL of the canine knee may have a more important role in knee stability than in humans. The flexed knee posture as well as the fact that the canine tibial plateau has a steeper posterior slope both tend to increase the forward thrust of the tibia on the femur. This may explain why there is a higher incidence of osteoarthritis after an ACL tear in dogs. Increased forward thrust increases the shear forces on the articular cartilage of the tibiofemoral joint that leads to the loss of this tissue.

There are many possible sources of error in this project. Gold chloride stain has been shown to color tissues containing neural elements. When using gold chloride there is always a possibility of the stain not working uniformly in the ligament. This occurred in some sections of our ligaments. This could have occurred selectively in the middle and distal thirds of the ligament for an unknown reason but it is unlikely. The fact that our ligaments were cut thinly allowed us to examine many areas at small intervals and the stain quality seemed in the most part homogeneous.

It is much easier to establish the presence or absence of a receptor than to try to precisely identify the type of receptor present. Although the qualification of the receptors was performed, distinguishing between the different types of receptors is difficult. Analysis of this data was not reliable enough to warrant statistical analysis. On the other hand, the presence or absence of receptors was accurately measured, therefore considered valid, and presented in this paper.

Another source of error is the small sample size. Even without performing statistical analysis on the raw data, it was obvious that there were many more receptors in the proximal portion of the ligaments. The division of the ligaments into thirds was an arbitrary decision. It was also performed in an arbitrary way. The ligament was sectioned into three parts of equal size but this was done by gross estimation and not according to precise measurements. This does not diminish the worth of this study, however, since the goal was to try and determine whether there was a real difference between the number of receptors present in different sections. Since the section was arbitrarily defined as a third, the millimeter difference that may have existed with a more precise measurement of each section would not have changed the significance of the results.

The significance of this study is that different parts of the ACL may contain a greater afferent source of information or proprioception than the rest of the ligament. This could result in a different perception of what type of ligament tear causes the patient the greatest degree of disability. Proximal ACL injuries may result in a greater loss of proprioception than a distal tear or avulsion. For the same reason, preservation of the proximal stump during reconstruction may increase the patient's knee proprioception. Therefore, reconstruction of the ligament may have to be modified to allow the proximal stump of the ligament to be sutured to the graft, thus improving the reconstructed ligament 's proprioception.

This study suggests that there are a higher number of mechanoreceptors in the proximal third of the canine ACL than in the rest of the ligament. These results cannot be extrapolated to humans. The role of mechanoreceptors in knee proprioception is still a matter of debate. This study provides us with some information that may be helpful in understanding the role of these receptors in the ACL but does not provide any definite conclusions.

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