Mechanoreceptors in the medial and lateral glenohumeral ligaments of the canine shoulder joint

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Summary
In order to have a better understanding of the role of ligaments in canine shoulder joint stability, the presence of mechanoreceptors in the medial (MGHL) and lateral (LGHL) glenohumeral ligaments was detected by means of a modified gold chloride stain. Three morphologically distinct mechanoreceptors were identified: Ruffini receptors (type I endings), Pacinian corpuscles (type II endings) and Golgi tendon organ-like receptors (type III endings). These receptors are mainly localized at each end of the ligaments and are prevalently in their glenoid portion. In particular, in the MGHL the highest density was at the cranial end of the insertion into the scapula. The variety of mechanoreceptors in canine shoulder ligaments might indicate an different function in providing the CNS with joint proprioceptive information. Therefore, besides acting as passive mechanical stabilizers, the MGHL and the LGHL may serve as sensory structures, contributing actively to joint stability. Ligamentous injuries which occur in shoulders not only affect mechanical restraint but also alter the proprioceptive input to the CNS by means of disruption of the mechanoreceptors.

Keywords
Mechanoreceptors, glenohumeral ligaments, shoulder joint stability, dog

Introduction
Shoulder joint stability is provided by various components: joint compression from the cuff muscles (1–3), negative intra-articular pressure (3, 4), the adhesion-cohesion mechanism by means of joint fluid (5) and by the joint capsule with its associated glenohumeral ligaments (6, 7). In the literature, many authors have placed emphasis on the importance of ligaments in joint stability. In fact, ligaments play a double role in joint stability: they act as a mechanical restraint (6, 8, 9) and, furthermore, as demonstrated electrophysiologically, they participate in an important reflex between the ligaments themselves and the cuff muscles (10–12). The reflex originates from the mechanoreceptors present within the ligaments. The mechanoreceptors are mechanically sensitive neuronal endings which transduce mechanical tissue deformation that has occurred either through voluntary movement or joint perturbation, as frequency modulated signals to the central nervous system through afferent proprioceptive pathways. Proprioception can be described as afferent information which typically arises from peripheral mechanoreceptors that contribute to postural control, joint stability and conscious sensation of movement. Sensation of movement can be further divided into joint position sense and the detection of passive movement sense. Collectively, these two sensory modalities are commonly referred to as joint kinaesthesia. In human medicine, knowledge about the presence and distribution of the mechanoreceptors and about the reflex has led to important findings regarding the basic concepts of treatment (13, 14), thus improving the understanding of some pathogenetic mechanisms of shoulder luxation. In fact, it has been demonstrated that shoulder instability is associated with proprioceptive deficits (15, 16).

Although shoulder joint stability is a topic of great interest among veterinary orthopaedists (6, 17–22), until now there have not been any neurohistological studies concerning the canine glenohumeral ligaments. Only a few studies involving mechanoreceptors in dog joints have been carried out on the knee joint (23, 24). Therefore, the present investigation was undertaken in order to ascertain whether canine medial (MGHL) and lateral (LGHL) glenohumeral ligaments contain mechanoreceptors, and in order to identify their type and localization in the hope of shedding light on their specific functions.

Materials and methods
The MGHL and LGHL from both sides of six fresh adult canine cadavers, weighing from 3–30 kg, and of different breeds, were obtained at necropsy. All of the shoulders were free from joint disease.

The ligaments were stained in bulk by using the gold chloride method of Gairns as modified by O’Connor and Gonzales (25). After staining, the tissue was dehydrated and paraffin-embedded according to standard histological procedures. The specimens were serially cut, 50 µm in thickness, along three different planes (sagittal, transverse and dorsal), in order to detect the three-dimensional distribution of mech-
anoreceptors in the ligaments. The sections were mounted on slides, coverslipped and observed with a light microscope (Zeiss, Axioplan). According to the Morisawa procedure, the following three conditions were required for the identification of the nerve endings: i) the specific morphology; ii) confirmation of the morphology in serial sections; iii) the presence of nerve fibres in the vicinity (26). The nerve endings were classified based on studies by Freeman and Wyke, (27) and Morisawa (26).

The images were recorded on a Polaroid DMC digital photo camera (Polaroid Corporation, Cambridge MA, USA) and DMC2 software and further processed by using Adobe Photoshop Element software. KS 300 Zeiss software (Kontron Elektronik, Eching, Germany) was used for morphometric analysis of the mechanoreceptors.

Results

Three morphologically distinct mechanoreceptors were identified both in the MGHL as well as in LGHL: Ruffini receptors or type I endings (Fig. 1), Pacinian corpuscles or type II endings (Fig. 2) and Golgi tendon organ-like receptors or type III endings (Fig. 3); in addition, free nerve endings or type IV endings (Fig. 4) were observed.

Ruffini receptors (type I endings)

These mechanoreceptors, globular or fusiform in shape, appeared singly or in small clusters of two to four end-organs. They were mainly found in the deeper layers of the ligaments. Each nerve ending consisted of a nerve fiber branched in a dense terminal arborisation wrapped in a thin capsule. The size of these receptors was variable, ranging from 46 to 173 µm in length, and 30 to 95 µm in width (average dimension: 115 x 58 µm).

Fig. 1  Ruffini receptors in LGHL (a, c) and MGHL (b, d). (b) The receptor partially wraps around a blood vessel (arrow). (c) A cluster of three type I endings. (a) and (b) bar = 100 µm; (c) bar = 200 µm; (d) bar = 50 µm. Gold chloride.

Fig. 2  Pacinian corpuscle in MGHL (a) and LGHL (b). Bar = 100 µm. Gold chloride.
In the MGHL, there were 1–16 type I endings per specimen (mean=SD: 7.6±5.9), which accounted for 51±19% of all the mechanoreceptors, whereas in the LGHL there were 1–5 type I endings per specimen (mean=SD: 3±1.3), which made up 46±12% of all the mechanoreceptors.

**Pacinian corpuscles (type II endings)**

These nerve endings were ellipsoidal corpuscles which averaged 250 µm in length and 45 µm in width. Each one consisted of a thinly encapsulated non-branching terminal nerve fibre. The single axon within the capsule was often slightly expanded and terminated near the tip of the corpuscle. Pacinian corpuscles were prevalently observed on the superficial layer of the ligaments and they were oriented parallel to the bundles of the collagen fibres.

In the MGHL, there were 1–5 type II endings per specimen (mean=SD: 2.9±1.5), which accounted for 29±17% of all mechanoreceptors, whereas in the LGHL there were 1–2 type II endings per specimen (mean=SD: 1.8±0.4), making up 35±17% of all the mechanoreceptors.

**Golgi tendon organ-like receptors (type III endings)**

These mechanoreceptors were large structures that measured approximately 1000 µm in length and 75 µm in width. They were innervated by a large afferent fibre which bifurcated into diverging branches. Each branch entered a thin capsule and gave off a dense arborisation which showed multiple small globular expansions. The long axis of these mechanoreceptors was found in the long axis of the ligament and, due to their dimensions, they occupied almost all the thickness of the ligaments themselves.

In the MGHL, there were 1–5 type III endings per specimen (mean=SD: 2.9±1.5), which accounted for 29±17% of all mechanoreceptors, whereas in the LGHL there were 1–2 type III endings per specimen (mean=SD: 1.8±0.4), making up 35±17% of all the mechanoreceptors.

**Distribution of mechanoreceptors in the ligaments**

The MGHL and LGHL were divided into thirds along the proximo-distal direction (glenoid, central and humeral portions) and the number of mechanoreceptors of each type was compared. In both ligaments, all of the receptors were mainly localized at each end of the ligaments and prevalently in the glenoid portion. In particular, in the MGHL, the percentage of type I endings in the glenoid, central and humeral portions was 81±16% (mean=SD%), 8±11% and 11±12%, respectively; the percentage of type II endings in the same portions was 84±22%, 7±19% and 9±16%, respectively; and the percentage of type III endings was 55±42%, 3±8% and 42±43%, respectively. Furthermore, in the glenoid portion of the MGHL, type I, type II and type III endings were most commonly found in the cranial branch of the insertion into the scapula, with this portion accounting for 88±15%, 79±35% and 55±30%, respectively, and in the caudal branch, with 12±15%, 27±35% and 45±30%, respectively.

In the LGHL, the percentage of type I endings in the glenoid, central and humeral portion was 57±33%, 19±24% and 24±27%, respectively, whereas the percentage of type II endings was 63±22%, 17±24% and 20±27%, respectively, and finally, the percentage of type III endings was 64±24%, 0±0% and 36±24%, respectively.

**Discussion**

To our knowledge, this study is the first investigation about the types and the topography of mechanoreceptors in the MGHL and LGHL of dogs. In this study, three types of specialized receptors were found: Ruffini receptors, Pacinian corpuscles and Golgi tendon organ-like receptors, and in addition, free nerve endings were also observed. This is in agreement with studies by other authors on human shoulder joints (26, 28, 29), mouse shoulder joints (30) and feline shoulder joint capsules (12), and limited to type I and type II endings in *Monodelphis*...
domestica shoulder joint (31). The most abundant mechanoreceptors found in dog shoulder ligaments were Ruffini receptors, followed by Golgi tendon organ-like receptors and Pacinian corpuscles. Our observations are generally consistent with those of Guanche et al. (29) and Steinbeck et al. (32) who described the Ruffini endings as the most common found in human glenohumeral ligaments. On the contrary, our data are different from the findings in the mouse (30) and Monodelphis domestica (31) shoulder joint region in which the Pacinian corpuscles were more abundant and the Ruffini receptors were identified only in small numbers.

With regard to the localization and distribution, the present study shows that most of the mechanoreceptors were located in the glenoid portion of the ligaments and, in particular, that the highest density was at the cranial arm of the MGHL. Previous investigations, in mouse (30) and Monodelphis domestica (31) shoulder joints, likewise reported that a large number of mechanoreceptors were located close to the glenoid labrum. In contrast, in human inferior glenohumeral ligaments, Ruffini endings were found close to the insertion on the humerus (32). The different distribution between canine and human models was predictable since the function of the canine shoulder joint differs considerably from the function of the human shoulder joint, for example, the canine shoulder carries a part of the body weight.

The variety of mechanoreceptors in canine shoulder ligaments might indicate an afferent function in providing the CNS with joint proprioceptive information. These specialized nerve endings most likely play an important role in the control of shoulder joint movement and stability. In fact, as demonstrated experimentally in the cat, a spinal reflex exists between the capsule-ligamentous structures and the muscles around the shoulder (10–12). Afferent proprioceptive information arising from the mechanoreceptors, which are located in the capsule-ligamentous structures, travels via the articular nerves to the spinal cord. The afferent neurons may synapse directly with the alpha or gamma motor neurons which innervate periarticular muscles or indirectly with interneurons (16, 32). Although there are many studies concerning the joint functions of specialized nerve endings, the role of this reflex arc has not yet been clarified. Early studies that demonstrated the existence of a reflex arc from the glenohumeral capsule to the cuff muscles, in anaesthetized cats, showed that a stimulation of the articular branches of the axillary, musculocutaneous, supraspinatus and subscapularis nerves elicited electromyographic activity in several muscles (biceps, subscapularis, supraspinatus, infraspinatus and deltoid) (10–12). The average latency of the muscular response to stimulation of the joint capsule was 3 msec, which was fast enough to be joint protective (10). The increase of periarticular muscle stiffness results in enhanced joint stiffness and, therefore, an augmentation of its stability (16). Recently, Diederichsen et al. (34) carried out a study in order to determine the effect of low amplitude electrical stimulation applied to the coracoacromial ligament (CAL) on the activity of shoulder muscles during isometric extension or flexion in conscious healthy humans. They demonstrated a general inhibition of the voluntarily activated shoulder muscles when the CAL was stimulated, with latencies ranging from 60 to 80 msec. These findings do not support the hypothesis of joint protective muscle reflexes but they do put forward the notion that these reflexes may contribute to muscle coordination and joint stability through a feedforward mechanism. Such results have previously been shown in the human shoulder after non-noxious stimulation of the glenohumeral joint capsule in conscious subjects (35). All of these data led us to hypothesize that in the dog, as in man and in cats, in addition to acting as passive mechanical stabilizers, glenohumeral ligaments may serve as a sensory structure by means of the mechanoreceptors embedded within them, contributing actively to joint stability.

The mechanoreceptors that are involved in the spinal reflex possess different physiological characteristics and functions; Ruffini and Golgi tendon organ-like receptors adapt slowly to movements, and identify motion and position of the ligament, thus allowing the CNS to interpret the position and the angle of rotation of the joint, whereas Pacinian corpuscles, which adapt rapidly to movements, can detect the acceleration and deceleration applied to the ligament at the beginning and the end of the movement (26–28, 36). Free nerve endings act as pain receptors, and also have a vasomotor action (27). The presence of a greater number of slowly adapting receptors with regard to quickly adapting receptors, suggests that the sensation of position and movement of the ligaments plays a more important role than the changes of velocity in the kinetics of the shoulder joint. This hypothesis must be confirmed by additional studies on shoulder kinetics but it could be explained when one considers that, whilst the chief movements of the canine shoulder are flexion and extension (37); this joint can move in any direction and the proprioceptive neural input from the shoulder requires a large number of receptors in order to continuously identify the position and the angle of rotation of the joint.

Moreover, our study showed that the highest density of these receptors was at the cranial arm of the glenoid portion of the MGHL. These observations led us to hypothesize that this end of this ligament is the most sensitive site, the one which receives the major part of the mechanical stimuli. These data could explain the large proportion of medial shoulder instability (75%) associated with an abnormal MGHL (19).

Furthermore, these findings may have some clinical implications. We can hypothesize that ligamentous injuries that occur in the shoulder from traumas or surgical procedures not only affect mechanical restraint but, because of the disruption of the mechanoreceptors, they can also alter the proprioceptive input to the CNS. For this reason, surgeons who perform shrinking or capsular shifts which involve capsuloligamentous structures in arthroscopic or open surgical procedures must take these effects into account and, when possible, they should avoid the mechanoreceptor-dense regions. However, it has been demonstrated that, in the rabbit, the mechanoreceptors regenerated after anterior cruciate ligament reconstruction using a free bone-patellar tendon-bone graft and, at eight weeks postoperatively, the number of receptors was not significantly different in the control nor in
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the grafted tendon (38). Georgoulis et al. (39) showed that in humans the mechanoreceptors present in the remnants of the ruptured anterior cruciate ligament are a possible source of re-innervation of the ligament autograft. Lephart et al. (14) demonstrated that at one year after thermal capsulorrhaphy, shoulder proprioception and function return to nearly normal in man. They speculated that the effects of rehabilitation may account for the normalized proprioception. In conclusion, a surgical procedure that preserves the glenohumeral ligaments as much as possible, as well as appropriate rehabilitation, can help to restore not only the mechanical restraint but also the original number, distribution and orientation of mechanoreceptors and, therefore, recovery from the proprioceptive deficit which results from a joint injury.

Further studies are warranted in order to investigate the presence and topography of mechanoreceptors in the whole canine joint capsule, as well as to detect the role of free nerve endings in the shoulder joint.

References


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