Muscle Pain: Understanding the Mechanisms

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Chapter 2
Functional Anatomy of Muscle: Muscle, Nociceptors and Afferent Fibers

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Contents

Abstract Nociceptors are free nerve endings, but not all free nerve endings in skeletal muscle are nociceptive. Nociceptive free nerve endings are connected to the CNS by thin myelinated fibers or unmyelinated afferent fibers. In the light microscope, free nerve endings look like a string of beads, i.e., they consist of axonal expansions (varicosities) connected by thin axonal segments. The neuropeptide substance P has been reported to be present predominantly in nociceptive afferent fibers.

In the electron microscope, a prominent feature of nociceptive nerve endings is that they are not free in the strict sense but ensheathed by Schwann cells.

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At present, there are no clear ultrastructural differences between non-nociceptive free nerve endings (e.g., sensitive mechanoreceptors and thermoreceptors) and nociceptive ones.

Functionally, different free nerve endings are assumed to possess different sets of receptor molecules in their axonal membrane. Receptor molecules that are particularly important for the function of muscle nociceptors are acid-sensing ion channels (ASICs) that open at a low tissue pH, P2X3 receptors that are activated by binding adenosine triphosphate (ATP), and the transient receptor potential receptor subtype 1 (TRPV1) that is sensitive to high temperatures and low pH.

2.1 Structure and Basic Function of Skeletal Muscle

A skeletal muscle is ensheathed by tight connective tissue, the epimysium, which in some muscles forms a dense fascia. An example is the anterior tibial muscle, which contracts inside the tube-like fascia with minimal distortion of the skin. Each muscle is composed of several fascicles (groups of muscle fibers or fiber bundles) that are surrounded by less dense connective tissue, the perimysium. The smallest macroscopic unit of a muscle is the muscle fiber (or muscle cell), each being separated from the others by a thin layer of loose connective tissue, the endomysium. Altogether, the connective tissue of a muscle can be viewed as a continuum that extends from the epimysium to the endomysium, and which has spaces in between that are filled by the muscle fibers. The connective tissue of muscle is functionally important, because it contains elastic fibers and maintains the shape and length of a muscle after deformation (contraction, stretch, pressure). Moreover, many pathologic processes take place not in the muscle cells proper but in the connective tissue where the blood vessels are located.

Each muscle fiber is composed of many myofibrils which consist of a chain of sarcomeres (Fig. 2.1). A sarcomere is the smallest functional unit of a skeletal muscle; it is approximately 2.5 μm (micrometers or microns) long in resting muscle. Inside the sarcomeres, the molecular filaments of actin and myosin are located, which interdigitate and slide against each other during contraction and relaxation of a muscle. The thin actin filaments are fixed to the Z band or Z disk that forms the border of a sarcomere in the direction of the long axis of the muscle cell. The myosin filaments are thicker than the actin filaments; they bridge the gap between two actin filaments. A third important molecule inside a sarcomere is titin, a long coiled molecule that is assumed to act like a spring, and brings the sarcomere back to its original length after stretch (Fig. 2.2). Titin also contributes to the elastic stiffness of a muscle.

The myofibrils in a muscle fiber are arranged in a way that the myosin and actin filaments of adjacent fibrils are in alignment. Thus, the myosin filaments of many myofibrils lie beside each other, and form the dark band (the anisotrope or A band) of the striations in skeletal muscle fibers that can be seen with a light microscope. Anisotrope means that the band is birefringent in polarized light. Without such an

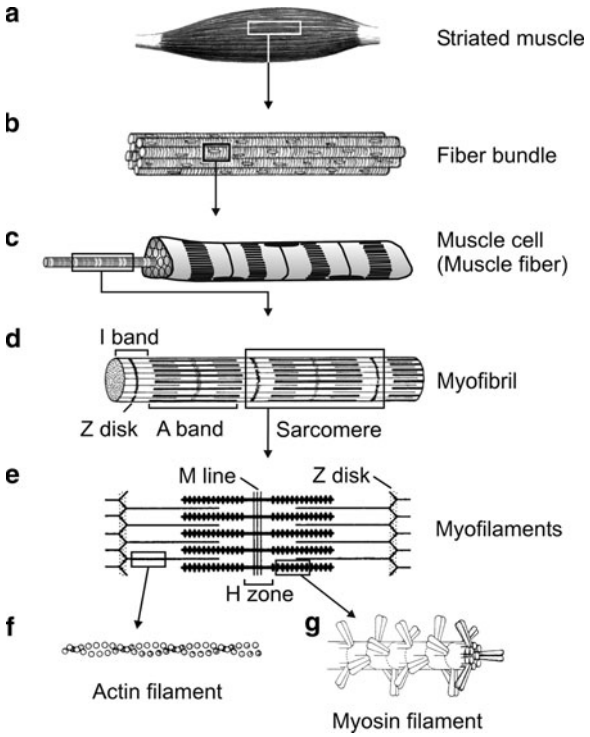

Fig. 2.1 Composition of skeletal muscle tissue. (**a**) Whole muscle showing longitudinally arranged muscle fiber bundles. The bundles are separated from each other by loose connective tissue (perimysium, not shown). (**b**) Muscle fiber bundle containing several muscle fibers (muscle cells). Each muscle fiber is enveloped by a fine layer of loose connective tissue (endomysium, not shown). (**c**) Single muscle cell showing the typical striations and three nuclei close to the cell membrane. The round profiles at the cut left end of the cell indicate cross-sections of myofibrils that contain the contractile filaments. One of the fibrils is shown protruding from the cell; it consists of a chain of sarcomeres. The *box* marks two sarcomeres that are shown in (**d**) at a higher magnification. Sarcomeres are the smallest functional units of a striated muscle; they extend from one Z line (or Z disk) to the next. (**e**) Components of a sarcomere. Thick myosin molecules with spiny heads lie in the center. They interdigitate with thin actin molecules that are fixed to the Z disk. The isotropic (I) band on both sides of the Z line contains actin filaments, only (see **d**). The anisotropic (A) band contains both actin and myosin filaments with the exception of its middle portion (the lighter H zone) which is free from actin. The M-line (M-band) consists of proteins that are important for the stability of the sarcomere structure; they crosslink the myosin filaments. During contraction, the actin and myosin filaments slide against each other; thus, the I band and H zone become narrower whereas the A band maintains a constant width. (**f, g**) An actin filament consists of two chains of globular proteins, whereas the myosin filament is a bundle of many threadlike proteins from which the myosin heads protrude. A single myosin molecule consists of two heavy protein chains and two pairs of light chains the latter forming the myosin head

accurate alignment of the fibrils – as is the case in smooth muscles – no striations would be visible. The filaments themselves can not be seen at the light microscopic level.

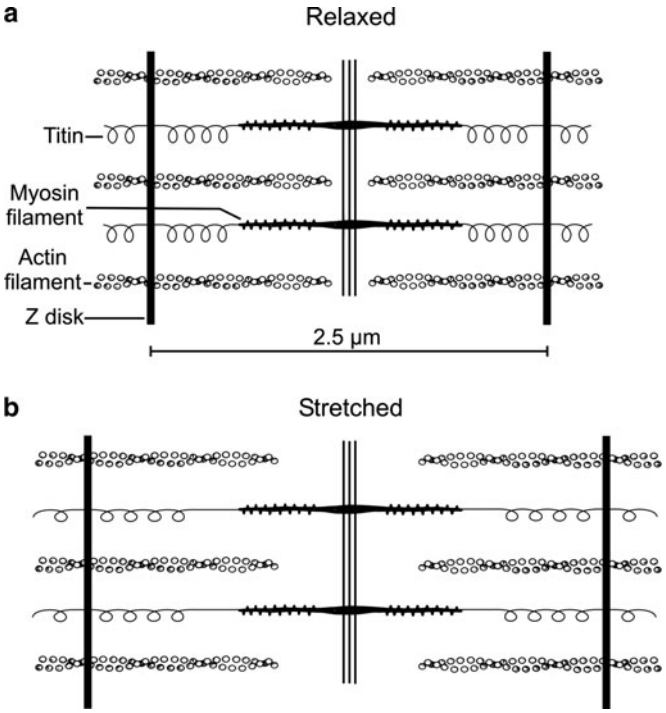

Fig. 2.2 The function of titin. Titin is the largest protein molecule of the human body; it consists of approximately 30,000 amino acids. The protein connects the myosin filaments with the Z disks (**a**) and has also connections with the actin filaments (not shown). It has a coiled structure, and functions as a spring that – together with the other elastic elements of muscle tissue – brings the sarcomere back to its original length after muscle stretch (**b**). Titin is probably an important factor contributing to the elastic stiffness of the muscle

The A band has a darker outer zone (closer to the Z disk) which is due to the overlap between myosin and actin filaments, and a paler inner zone, the H band or zone which marks that region of the A band where only myosin filaments are present. Sarcomere regions close to the Z disk contain actin filaments only; they look pale in the light microscope and form the isotropic (I) band (Fig. 2.1).

The contraction of a skeletal muscle cell is initiated by an action potential that arrives via the α-motor fiber at the neuromuscular endplate or junction. The action potential releases acetylcholine (ACh) from the terminal branches of the motor fiber (Fig. 2.3). The ACh diffuses across the synaptic cleft to the muscle cell membrane, and binds to specific receptor molecules on the surface of the membrane. The ACh binding opens ion channels, and positive ions (mainly Na^+) enter the muscle cell. This causes a depolarization of the muscle cell membrane, i.e., makes the inner side of the muscle cell membrane more positive. The depolarization is called endplate potential (EPP). Normally, the amount of ACh molecules released by a single action potential is large enough to depolarize the muscle cell membrane beyond firing threshold, and elicits an action potential. The normally suprathreshold potential at

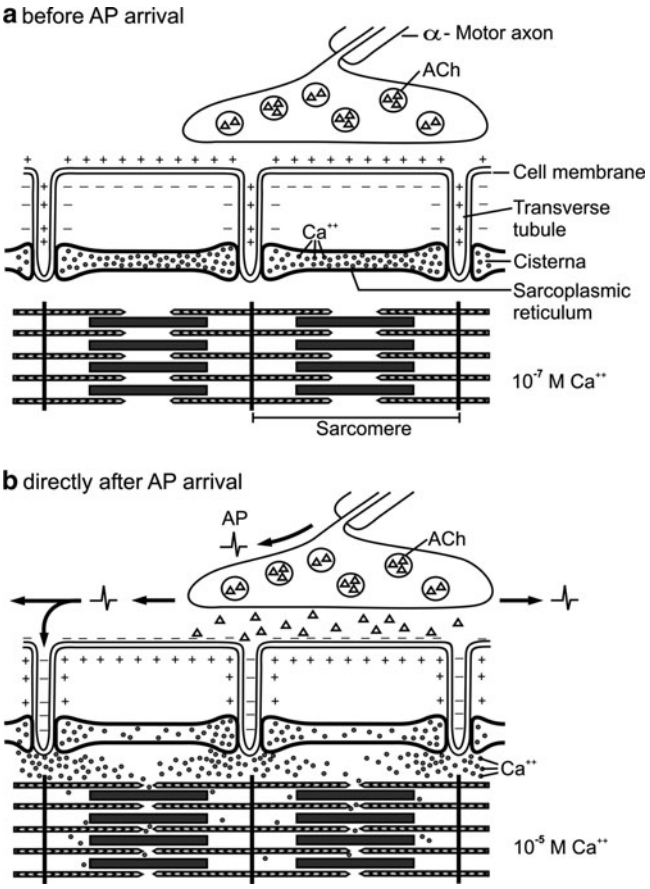

Fig. 2.3 Events leading to the sliding of the contractile filaments. The α-motor axon forms a presynaptic expansion, the neuronal part of the neuromuscular junction or endplate. The vesicles in the expansion contain acetylcholine (ACh, *open triangles*), the transmitter substance of the neuromuscular junction (**a**). When an action potential of the motor axon arrives at the endplate, it causes fusion of the vesicles with the presynaptic membrane and release of ACh into the synaptic cleft. The ACh molecules diffuse to the postsynaptic membrane, and bind to ACh receptor molecules in the membrane of the muscle cell. This induces opening of membrane channels, and influx of positively charged ions into the muscle cell. The result is a depolarization of the postsynaptic membrane, i.e., the negative electric charges on the inner surface of the muscle cell membrane (**a**) are replaced by positive ones. Normally, the postsynaptic depolarization induced by a single action potential in the α-motor axon is large enough to elicit an action potential in the membrane of the muscle cell adjacent to the endplate (**b**). The action potential is propagated over the entire muscle cell, enters the invaginations of the cell membrane (the transverse tubule) and releases calcium ions (Ca^{2+}) from the cisternae of the sarcoplasmic reticulum, which functions as an intracellular calcium store. The resulting increase in intracellular calcium concentration (from approximately 10^{-7}–10^{-5} M) is the signal for the myosin filaments to pull at the actin filaments by rowing movements of the myosin heads, and thus shorten the sarcomere (see Fig. 2.4). The ACh molecules in the synaptic cleft are cleaved by acetylcholine esterase which is present in the cleft. If the number of accessible postsynaptic ACh receptors is reduced by curare, which blocks the receptors, the depolarization does not reach firing threshold, and the muscle cell does not contract

the neuromuscular endplate is a clear difference to the postsynaptic potentials in CNS neurons, which are typically subthreshold.

In the muscle cell membrane, the EPP elicits a pair of action potentials that travel along the muscle cell in opposite directions. They make sure that all sarcomeres of the muscle cell contract at about the same time. The action potentials also reach the inside of the cell by following the transverse tubules. Transverse tubules are invaginations of the cell membrane; they have close contact with the sarcoplasmic reticulum (SR). The SR consists of a network of branching and anastomosing tubules that fill the space between the myofibrils. The reticulum has terminal expansions (cisternae) close to the transverse tubules which function as calcium stores of the muscle cell. The action potentials that enter the transverse tubules release Ca^{2+} from these cisternae. This leads to an increase of the Ca^{2+} concentration in the cytoplasm outside the SR from about 10^{-7} M to approximately 10^{-5} M. The high Ca^{2+} causes the molecules troponin and tropomyosin – which mask the binding sites for myosin on the actin filament in resting muscle – to change their positions on the actin molecule and unmask the binding sites (Fig. 2.4). Now, the heads of the myosin filaments can bind to actin, and as soon as they have done so, the neck of the myosin molecule makes a flexing movement. The sequence: attachment of the myosin heads to actin – flexion of the myosin neck – detachment of the myosin head – attachment of the myosin head to another site of the actin molecule results in a "rowing" movement of the myosin heads as long as the high Ca^{2+} concentration is present in the cytoplasm. The rowing movements pull the actin filament in between the myosin filaments, thus shortening the sarcomere.

The myosin heads possess an ATP-binding site and contain ATPase, an enzyme that splits adenosine triphosphate (ATP) into adenosine diphosphate (ADP) and a phosphate group (P) plus energy. ATP is present in the cytoplasm, and in resting muscle is bound to the myosin head, which in this state cannot attach to the actin molecule. The enzyme ATPase cleaves ATP, and the myosin–ADP-P complex can bind to actin, if the binding sites are made available by high Ca^{2+}. The binding process is followed by the release of P, which results in a flexing of the myosin head (the so-called power stroke which moves the actin to the center of the sarcomere). The attached myosin head binds ATP again, the myosin–ATP complex is detached from the actin, the ATP is cleaved by ATPase, and the neck of the myosin head returns to its initial angle. This cycle continues as long as enough ATP is available and the Ca^{2+} is high enough. Please note that the energy released by the ATP molecule is not used for the shortening process as such (the flexing of the myosin heads) but for changing the angle of the neck of the myosin heads back to the unflexed position, and for the detachment of the myosin heads from the actin). If after detachment of the myosin head the cytoplasmic Ca^{2+} concentration is still high, the heads can attach again to the actin filament. Many successive cycles of this attaching–flexing–separating process lead to a movement of the actin filament along the myosin. Since the actin filaments are attached to the Z disk, the sarcomere is shortened.

When the cytoplasmic calcium concentration drops to the initial value, the rowing movements of the myosin heads stop. The drop in Ca^{2+} is due to the action of a molecular calcium pump which transports the Ca^{2+} back into the SR. After

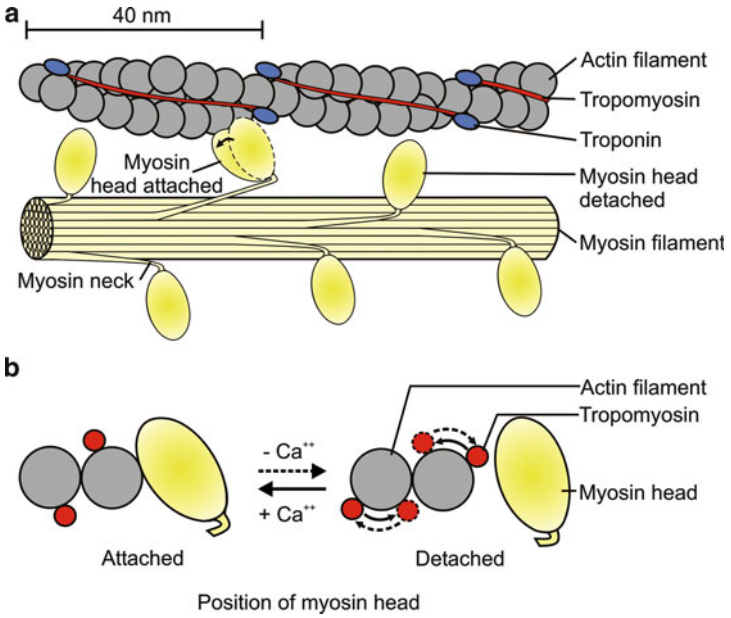

Fig. 2.4 The "sliding filament" mechanism during contraction. In the resting state, the binding sites for the myosin heads on the globular molecules of the actin chain are masked by the threadlike tropomyosin molecule and therefore not accessible (**a, b**). When an action potential of the muscle cell releases Ca^{2+} from the sarcoplasmic reticulum and the Ca^{2+} concentration in the cytoplasm of the cell rises, the tropomyosin that covers the binding sites moves aside, and the myosin heads attach to the actin molecules (**b**). Subsequently, the neck of the myosin heads make a bending movement, and thus pull the myosin filament towards the Z line (**a**). Since the attached myosin heads have the action of an ATPase (an enzyme splitting adenosine triphosphate, ATP), energy-rich ATP molecules are cleaved. The released energy is used for separating the myosin heads from the actin. By repeating this sequence of attaching, bending, and separating, the myosin heads make a rowing movement which leads to the sliding movement of the filaments that shortens the sarcomere

death, there is a general lack of metabolic energy, and the cytoplasmic ATP concentration is very low. Therefore, the myosin heads cannot separate from the actin, and rigor mortis ensues. In rigor mortis, all myosin heads are attached to actin, a situation which never occurs during life because the myosin heads take turns and some heads are always not attached.

In the microscope, a contracted muscle fiber can be recognized by the unchanged width of the A band and a narrower I and H band. There is evidence in the literature showing that in resting muscle a small proportion of the myosin heads are attached to the actin filaments (Campbell and Lakie 1998). These attached myosin heads probably contribute to the viscoelastic component of muscle tone (see Chap. 6).

Physiologically, a skeletal muscle can perform four types of contraction.

1. *Shortening (concentric) contraction*. This is characterized by reduction in muscle length produced by a generation of muscle force. An example is the

quadriceps femoris muscle extending the knee during walking uphill. These contractions, which are characterized by simultaneous length and force changes, have been termed auxotonic contractions (Li and Stephens 1994). Most contractions performed in daily life are auxotonic.

2. *Isotonic contraction.* This is defined as length change without change in the force exerted. Isotonic contractions are rare in the normal environment, because almost all movements are associated with a change in force. For instance, when lifting an arm without any load, the force of gravity, against which the contraction has to be performed, will increase until a horizontal position is reached and then decrease again. Pure isotonic contractions can be performed on an exercise machine which provides constant resistance through the range of movement.
3. *Isometric contraction*. The term describes an increase in force without length change. Isometric contractions are rare in daily life. An example is activation of the masseter muscle when the maxillary and mandibular teeth are in contact. The teeth and their supporting tissues have limited compressibility; therefore, the contraction is largely isometric. Under these conditions, the myosin heads perform their movements and pull at the actin filament, but the developed force is used not for shortening of the muscle but for putting tension on the insertion points and for stretching the elastic tissue components of the muscle. Thus, the sarcomeres shorten, but the muscle as a whole does not.
4. *Lengthening (eccentric) contraction*. This is defined as a lengthening of muscle by external forces, with the muscle resisting the lengthening. In eccentric contractions, the force developed by the muscle is smaller than that causing the lengthening (otherwise the muscle would not be lengthened). The muscle contracts to slow the lengthening. An example is the contraction of the quadriceps muscle during walking downhill. This type of contraction is particularly important for the development of muscle soreness (see below).

Skeletal muscle is composed of two main types of muscle fibers, namely "white" and "red" fibers. The proportion of each fiber type varies between muscles. White fibers look pale because they contain less myoglobin than red ones. They contain large amounts of phosphorylases and glycogen, and produce energy mainly by degrading glucose, i.e., they obtain energy by the glycolytic metabolism. One of the end products of the glycolytic metabolism is lactate, which after a short bout of contractions accumulates in such an amount that the tissue pH drops and the work has to be terminated, i.e., white fibers fatigue quickly. When white fibers are activated by a short impulse, they contract in the form of a fast twitch (approximately 25 ms duration). They are used in movements of high velocity and short duration. Examples are flight reactions of animals or short distance running in humans. The gastrocnemius muscle is a typical white muscle that is used for the fast movements of the legs.

Red muscle fibers contain more myoglobin and oxidative enzymes but less phosphorylases. When activated, they contract with slow twitches (75 ms duration), and are more resistant to fatigue. They are able to contract for long periods, because

they make use of the oxidative metabolism, i.e., they consume oxygen and obtain ATP from the mitochondria. Ideally, red fibers degrade glucose to CO_2, water, and energy. Fibers of this type are numerous in postural muscles, because they are well-suited for slow contractions of long duration. The true muscles of the back are typical examples of muscles with a high proportion of red fibers. The white fibers correspond largely to Type II fibers, and the red fibers to Type I fibers.

Small lesions of skeletal muscle can be repaired by satellite cells that are present underneath the cell membrane of each muscle cell. They are assumed to be myoblasts, i.e., cells that develop into muscle cells during development. The muscle fibers formed by these cells during regeneration of a damaged muscle are indistinguishable from the original ones. Larger areas of damaged muscle can not regenerate; they are replaced with connective tissue.

2.2 Morphology of Muscle Nociceptors

The fact that small-diameter afferent fibers have to be activated in order to elicit muscle pain is well-established (Weddell and Harpman 1940). These fibers conduct at a velocity of below 20–30 m/s (depending on the species studied), which is relatively slow in comparison to the fastest conducting fibers, which reach 100 m/s. Histologically, they consist of thin myelinated (group III) and nonmyelinated (group IV) fibers. The conduction velocity of group IV fibers is approximately 0.5–2.5 m/s in the cat, that of group III, 2.5–30 m/s. The nomenclature with Roman numerals (group I–IV) was introduced by Lloyd (1943) for muscle afferent fibers, but is now generally used for fibers from deep somatic tissues in general (muscle, joint, fascia, tendon, ligaments). Group III fibers correspond to cutaneous Aδ-, and group IV to C fibers. It is worth mentioning that not all of these small-caliber or slowly conducting fibers are nociceptive; they also include thermoreceptive and mechanoreceptive fibers. Therefore, the terms "slowly conducting fibers" or "small-caliber fibers" must not be used as synonyms for "nociceptive."

In cutaneous nerves, there are thermoreceptors and low-threshold mechanoreceptors that have unmyelinated afferent fibers, and low-threshold mechanoreceptors with group IV afferents have also been found in skeletal muscle (Light and Perl 2003; Hoheisel et al. 2005). The presence of certain neuropeptides such as CGRP does not distinguish between high- and low-threshold mechanosensitive group IV-fiber units, because the neuropeptide is found in both functional types (Hoheisel et al. 1994).

2.2.1 Structure of Muscle Nociceptors in the Light and Electron Microscope

The first comprehensive report on the morphology of free nerve endings in skeletal muscle was published by Stacey (1969). He focused on endings supplied by

group III and IV fibers. As mentioned above, nociceptors have the morphological appearance of free nerve endings. The term "free nerve ending" indicates that in the light microscope no (corpuscular) receptive structure (Stacey 1969) can be recognized. When immunohistochemical staining for neuropeptides is used, the receptive ending looks like a string of beads, with the beads (so-called varicosities) having relatively wide diameter connected by very thin stretches of axon. The diameter of a branch of a free nerve ending is 0.5–1.0 μm, i.e., at the limit of the spatial resolution of the light microscope (Fig. 2.5a, b). A receptive ending together with its afferent fiber is called an "afferent unit."

In Stacey's study, the majority of the group IV fibers had a diameter of approximately 0.35 μm. The unmyelinated afferents were numerous and outnumbered the myelinated ones by a factor of 2. The predominant location of free nerve

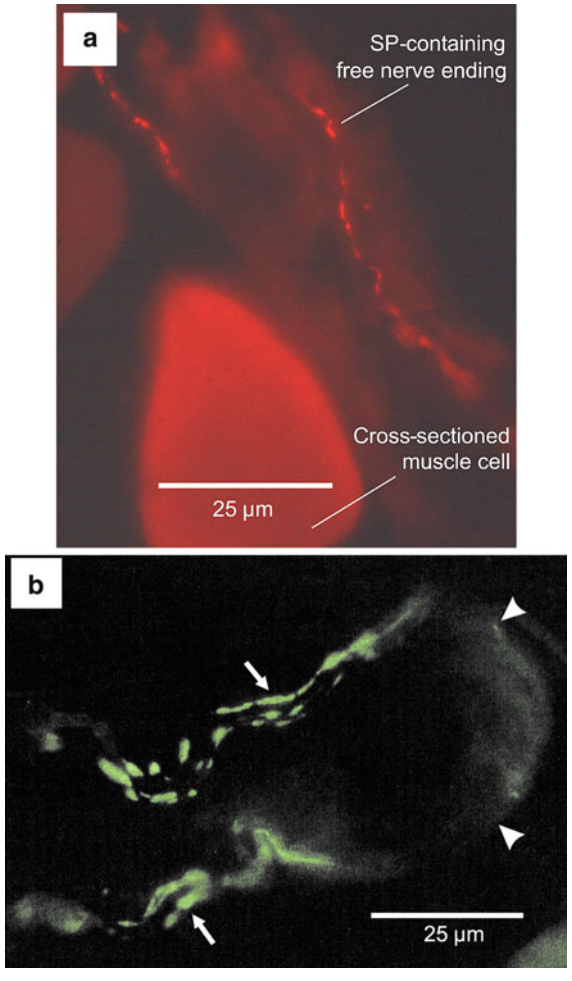

Fig. 2.5 Histology of free nerve endings in muscle. Photographs of histological sections of the rat gastrocnemius muscle show free nerve endings that contain the neuropeptide substance P (SP, **a**) and calcitonin gene-related peptide (CGRP, **b**) respectively. The fibers were visualized using antibodies to SP (**a**) or CGRP (**b**) coupled to a fluorescent marker. Please note the typical appearance of the endings as a string of beads with localized widenings of the axon (so-called varicosities) that contain vesicles filled with SP- or CGRP-immunoreactive material. From these varicosities, the neuropeptides are released when the nerve fibers are electrically active. The CGRP-containing ending was located in the connective tissue around an arteriole (*arrowheads*), the typical location of free nerve endings in muscle

endings supplied by group IV fibers was the adventitia of arterioles and venules. Surprisingly, muscle fibers themselves did not receive direct innervation by free nerve endings. Group III afferents generated not only free nerve endings but also paciniform corpuscles, whereas group IV fibers terminated exclusively in free nerve endings. The high sensitivity of the free nerve endings to chemical stimuli, particularly to those accompanying inflammatory lesions or disturbances of the microcirculation, may be related to their location on or in the walls of the blood vessels. The finding that the muscle fibers proper are not supplied by free nerve endings (Reinert et al. 1998) may relate to the clinical experience that muscle cell death is usually not painful, at least not if it occurs slowly, as for instance during muscular dystrophy, polymyositis, or dermatomyositis. A different situation is tearing of a muscle fiber bundle, which can be extremely painful. In this condition, many muscle cells are destroyed simultaneously and release their contents (e.g., K^+ ions and ATP) in the interstitial space, from where they can diffuse to the next nociceptive endings.

Usually, a single group IV fiber has several branches, each of which possesses various receptive sites. All branches that are located closely together in a small volume of tissue form a receptive ending. An ending can extend over relatively long distances and can have several receptive branches which again have several receptive sites. The physiological term receptor refers to an entire morphological ending.

At present, it is not possible to correlate morphological features of free nerve endings with the functional types found in electrophysiological experiments (see Chap. 3). There is general agreement that muscle nociceptors are free nerve endings, but their exact ultrastructure is unknown and no electronmicroscopic criterion exists that allows a distinction between a nociceptive free nerve ending and a thermoreceptive or mechanoreceptive one. However, the notion that there are several morphological types of free nerve ending in muscle supports the assumption that these receptors do not form a homogeneous group, but consist of functionally different types.

In skeletal muscle, the free nerve endings appear to be distributed quite evenly in the proximodistal direction. At least, in a quantitative evaluation of the innervation density by neuropeptide-(SP- and CGRP-) containing fibers, no difference between the proximal and distal portions of the rat gastrocnemius–soleus (GS) muscle was found (Reinert et al. 1998). Therefore, a higher innervation density at the transition zone between muscle and tendon is not a probable explanation for the frequent pain in this region. However, in the same study the nerve fiber density in the peritendineum (the connective tissue around a tendon) of the rat calcaneal tendon was found to be several times higher than that in the GS muscle. In contrast, the collagen fiber bundles of the tendon tissue proper were almost free of free nerve endings. The high fiber density in the peritendineum may explain the high prevalence of tenderness or pain in the tissue around the tendon and the insertion site. The scarcity of nerve endings in the center of the tendon may relate to the clinical observation that (incomplete) ruptures of the tendon may occur without pain.

In an electron microscopic investigation on sympathectomized cats, von Düring and Andres (1990) reported that group III and IV endings were predominantly located in the perimysium surrounding larger or smaller bundles of muscle fibers. Other locations were the adventitia of arterioles, venules, and lymphatic vessels, and finally the sheath of nerve fiber bundles. These latter terminals were assumed to originate from nervi nervorum (nerves supplying the nerves themselves). In muscle, the terminals of group III fibers were generally larger than those of group IV fibers, and they contained more mitochondria and a more distinct receptor matrix (see below). The authors suggested that those terminals (mainly originating from group III fibers) that had a close association with connective tissue may have a mechanoreceptive function mediating stretch or pressure, whereas endings that lacked this feature, but had a spatial relation to mast cells, were nociceptors.

Electronmicroscopic data demonstrate that the varicosities of free nerve endings contain mitochondria and vesicles, and show other structural specializations characteristic of receptive structures. However, free nerve endings are not free in the strict sense, because most of them are ensheathed by a single layer of Schwann cells. In contrast to myelinated fibers where each Schwann cell is wrapped in multiple layers around a small stretch of the axon, the Schwann cells of unmyelinated fibers form a single layer. The Schwann cells of unmyelinated fibers leave parts of the axon membrane uncovered (Andres et al. 1985). Here, the only structure that separates the axon membrane from the interstitial fluid is the basal membrane. The exposed membrane areas are assumed to be the sites where external stimuli (particularly chemical stimulants) act (Fig. 2.6; Andres et al. 1985; Messlinger 1996; see also Heppelmann et al. 1990a, b for free nerve endings in the joint).

The arrangement of cell organelles [mitochondria, vesicles, and axonal reticulum (a network of fluid-filled vacuoles or channels)] embedded in the axonal cytoplasm of the varicosities was called *receptor matrix* by Andres and von Düring (1973)) (see also Kruger et al. 2003). Often, nociceptive endings exhibit granular or dense core vesicles containing neuropeptides. The function of the round clear vesicles in the peripheral ending is still obscure. They may contain the same transmitters as the central synaptic terminal, which is glutamate for nociceptive afferent units.

2.2.2 *Receptor Molecules in the Membrane of Nociceptors*

In the following paragraphs, a distinction is made between "receptor" as a term for a receptive nerve ending, and "receptor molecule" (or "membrane receptor") for a molecule that binds a specific stimulant or is activated by a thermal or mechanical stimulus. In the biochemical literature, the term "receptor" describes the receptor molecule.

The specific receptor molecules in the membrane of nociceptive endings (Fig. 2.7) are divided into two main classes: ionotropic and metabotropic receptors.

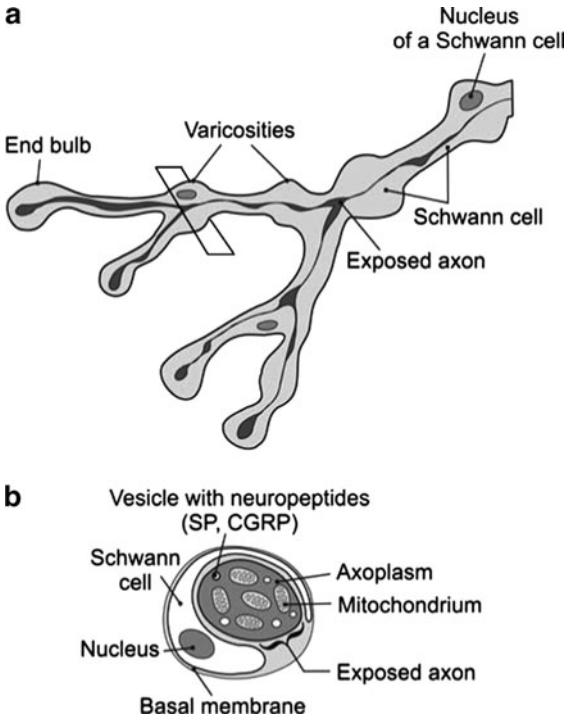

Fig. 2.6 Ultrastructure of free nerve endings. (**a**) Reconstruction of a free nerve ending from electron microscopic sections. The ending has several branches and exhibits the typical varicosities. Schwann cell processes ensheath the ending almost completely, and leave only small patches of axon uncovered (exposed axon areas). The exposed areas are the site where chemical stimuli are assumed to act. (**b**) Cross-section through a varicosity of the ending (*boxed* in **a**). The varicosity resembles a presynaptic terminal in that it contains many mitochondria (providing energy) and vesicles containing neuropeptides that are released when the ending is excited

Ionotropic receptors are large proteins that form a channel or pore that spans the entire width of the axonal membrane. Usually the channel is closed; after binding a specific stimulating molecule (its ligand), the channel opens, and ions flow across the membrane according to their concentration difference inside and outside the membrane (Na^+ ions will enter the ending, K^+ and Cl^--ions leave the ending). Binding of the ligand to a metabotropic receptor on the outer surface of the membrane activates a G protein (guanine nucleotide-binding protein) on the inside of the membrane. G proteins alternate between an inactive guanosine diphosphate (GDP) and active guanosine triphosphate (GTP) bound state; in the activated state, they regulate intracellular metabolic cascades. For instance, they change the state of activation of intracellular second messenger systems such as phospholipase C (PLC), cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), and protein kinases (PKs). One sequel of the activation of these messengers, particularly the PKs, is the phosphorylation of ion channels. Phosphorylation means that a phosphate residue is coupled to another molecule, for instance a channel protein. The phosphorylation increases the opening time or opening probability of the channels, i.e., it leads to increased ion fluxes across the membrane. Collectively, the processes lead either to direct excitation of the ending or to a changed reaction of the nerve ending to external stimuli [sensitization (increased excitability) or desensitization (decreased excitability)].

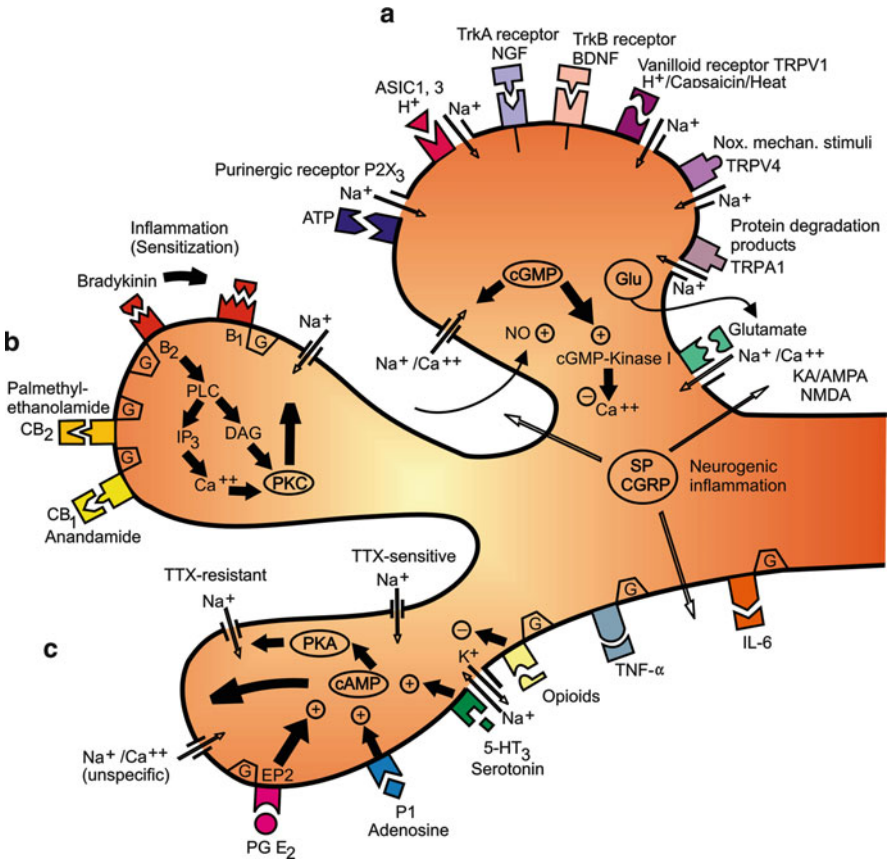

Fig. 2.7 Membrane receptors of a nociceptive nerve ending. Of particular importance for muscle pain are the receptor molecules and processes shown in branches **a**, **b** and **c**. (**a**) There are two main receptors sensitive to H^+ ions: acid-sensing ion channels (*ASIC 1, 3*) and the transient receptor potential subtype V1 (*TRPV1*). The purinergic receptor *P2X3* binds *ATP*, a molecule that is present in each cell of the body but has a particularly high concentration in muscle cells. ATP could serve as a general pain signal, because it is released by any cell damage. All these receptor molecules are ion channels which span the axonal membrane and are permeable to Na^+ and other cations (Ca^{2+} and/or K^+). (**b**) Shows the change of the bradykinin receptor B2 to B1. In intact tissue, *bradykinin* (BKN) excites or sensitizes the ending by acting on the B2 receptor; in inflamed tissue, it binds to the newly synthesized B1 receptor. BKN exerts its action not by opening an ion channel but by activating a G protein that regulates intracellular metabolic changes. These changes lead to an increased excitability of the ending (sensitization). The change from B2 to B1 shows that even in the very periphery there are neuroplastic changes, i.e., functional or morphological changes of neurons brought about by pathological tissue alterations. (**c**) In addition to BKN there are other sensitizing substances such as prostaglandin E2 (PGE_2) and serotonin (*5-HT*) which likewise bind to specific membrane receptors. The receptors for 5-HT_3 and PGE_2 induce intracellular cascades of events that increase the sensitivity of the Na^+ channels by activating protein kinase A (*PKA*). The larger ion currents that flow through the channel proteins of a sensitized ending render the ending more sensitive to external stimuli. Branch c also shows that tetrodotoxin(TTX)- resistant Na^+ channels are present in the membrane of nociceptive endings. *cAMP*, cyclic adenosine monophosphate, a second messenger

Figure 2.7 shows a large number of receptor molecules that have been detected in the membrane of nociceptors in a variety of tissues. Judging from their responsiveness to pain-producing agents, the following receptor molecules are likely to be relevant for muscle pain and tenderness (Mense and Meyer 1985; Caterina and David 1999; McCleskey and Gold 1999; Mense 2007).

Bradykinin (BKN) receptors (B1 and B2). BKN is cleaved from blood plasma proteins when a blood vessel breaks or increases its permeability so that plasma proteins enter the interstitial space. In intact tissue, BKN excites nerve endings by the activation of the BKN receptor molecule B2, whereas under pathological conditions (e.g., inflammation) the receptor B1 is the predominant one (Perkins and Kelly 1993; for a review of receptor molecules mediating the effects of classic inflammatory (pain-producing or algesic) substances, see Kumazawa 1996). This means that in a pathologically altered muscle, for instance during inflammation, the damaged tissue sends a signal (probably nerve growth factor (NGF)) to the cell body (the soma or perikaryon) of the primary afferent neuron, which then synthesizes B2 receptors that are transported in the cytoplasma of the axon to the receptive ending and built into its membrane. The de-novo synthesis of a receptor molecule is a neuroplastic change; it demonstrates that neuroplastic changes occur not only in the CNS but also in the peripheral afferent neuron. It is often overlooked that many of the so-called pain-producing substances such as BKN excite not only nociceptors but also low-threshold mechanosensitive (presumably non-nociceptive) endings. Therefore, BKN cannot be considered a specific excitant of nociceptors.

Serotonin receptors (particularly 5-HT_3). Serotonin (5-hydroxytryptamin, 5-HT) is released from blood platelets during blood clotting. The stimulating effects of serotonin on nociceptive terminals in the body periphery are predominantly mediated by the 5-HT_3 receptor (at present, more than 15 different 5-HT receptors are known in the CNS). The serotonin concentrations released in the tissue are usually not sufficient to excite nociceptors directly, but they can sensitize them, i.e., make them more sensitive to other pain-producing agents such as BKN.

Prostaglandins, particularly prostaglandin E2 (PGE_2). Prostaglandins (PGs) are released in a pathologically altered muscle by the enzymatic action of cyclooxigenases. PGE_2 binds to a G protein-coupled prostanoid receptor (EP2) in the membrane of the nociceptive ending. Similarly to serotonin, PGE_2 sensitizes nociceptors rather than exciting them under (patho)physiological circumstances (Mense 1981).

Acid-sensing ion channels (ASICs). ASICs constitute a family of receptor molecules that are sensitive to a drop in pH and open at various pH values. The channel proteins react already to small pH changes, for instance from pH 7.4 to 7.1. This receptor family (for instance ASIC1 and ASIC3) is particularly important for muscle pain, because almost all pathologic changes in muscle are accompanied by a drop in tissue pH, e.g., exhausting exercise, ischemia, and inflammation (Immke and McCleskey 2003). In these conditions, the pH of the muscle tissue can drop to 5–6. The proton-sensitive nociceptors may also be of

importance for the induction of chronic muscle pain. Repeated intramuscular injections of acidic solutions have been reported to induce a long-lasting hyperalgesia (Sluka et al. 2001).

P2X3 receptors. This receptor is a subtype of the purinergic receptors that are activated by ATP and its derivatives (Burnstock 2007; Ding et al. 2000). ATP is the energy-carrying molecule in all cells of the body; accordingly, it is present in every tissue cell. It is released from all tissues during trauma and other pathologic changes that are associated with cell death. For this reason, ATP has been considered a general signal substance for tissue trauma and pain (Cook and McCleskey 2002). ATP is particularly important for muscle pain, because it is present in muscle cells in high concentration (Stewart et al. 1994). When injected into human muscle, ATP causes pain (Mörk et al. 2003).

Transient receptor potential receptor subtype 1 (TRPV1) formerly called VR1. This receptor is one of the most important molecules for the induction of pain. The natural stimulant for the TRPV1 receptor is Capsaicin, the active ingredient of chilli peppers (Caterina and Julius 2001). The receptor is also sensitive to an increase in H^+-concentration and to heat, with a threshold of approximately 39°C. Its endogenous ligands are H^+-ions. As for ASICs, the sensitivity of this receptor to protons is important under conditions in which the pH of the tissue is low. Acidity of the tissue increases the sensitivity of the receptor molecule to heat. For instance, in tissue with a pH of 6.3, a temperature of 26°C is sufficient for activating the receptor. Under these conditions, the normal body temperature can cause pain (Reeh and Kress 2001). This finding offers an explanation for the aching of the whole body in general infections such as flu, where the body temperature is raised (and the tissue pH probably lowered).

In microneurographic recordings from muscle nerves in humans, muscle nociceptors have been found that could be activated by i.m. injections of capsaicin (Marchettini et al. 1996). The capsaicin injections were associated with strong muscle pain. Since capsaicin is assumed to be a specific stimulant for the TRPV1 receptor, these data show that this receptor molecule is also present in human muscle nociceptors.

Other TRP receptors. TRPV4 is a mechanosensitive ion channel that is sensitive to both weak and strong (noxious) intensities of local pressure (Liedtke 2005). It may be the receptor for mediating pain evoked by pinching and squeezing. Another candidate for this function is the degenerin/epithelial sodium channel (DEG/ENaC; Goodman et al. 2004). TRPA1 has been reported to mediate pain elicited by protein degradation products that are present during many pathological tissue alterations, and during experimental induction of a tissue lesion, e.g., by injection of formaldehyde (Macpherson et al. 2007). The TRPA1 receptor is considered by some to be the central molecule for chemically induced pain (Tai et al. 2008).

Tyrosine kinase A (TrkA) receptor. The ligand of this receptor is NGF (Caterina and David 1999). NGF is well-known for its sensitizing action on nociceptors in the body periphery and neurons in the CNS; it is synthesized in muscle, and its synthesis is increased during pathophysiological changes of the muscle (e.g., inflammation, Menetrey et al. 2000; Pezet and McMahon 2006).

Glutamate receptors. There is evidence indicating that the NMDA receptor (one of the receptors for glutamate) is present on nociceptive endings in masticatory muscles. Injections of glutamate into the masseter muscle in human subjects induced a reduction in pressure pain threshold which was attenuated by coinjection with ketamine, an NMDA receptor antagonist (Cairns et al. 2006). The glutamate-induced mechanical sensitization did not spread outside the injection site; therefore, the glutamate effect likely resulted from a peripheral mechanism, i.e., from an action of glutamate on peripheral NMDA receptors.

Substances exciting muscle nociceptors independent of membrane receptors.

Hypertonic saline: injections of NaCl solutions (4.5–6.0%) have long been and still are used to elicit pain from deep somatic tissues (Kellgren 1938; for review, see Graven-Nielsen 2006). Single injections or infusions of the hypertonic solution reliably elicit a medium level of pain in patients and healthy controls. The traditional explanation for the pain-eliciting action of hypertonic saline is that when injected in high concentrations, Na^+ ions enter the ending through sodium channels and reduce the negative potential on the inside of the axonal membrane. Recently, the notion that hypertonic solutions excite nociceptors through an unspecific mechanism (e.g., by osmotic volume changes of the receptive ending) has been challenged, because there are data showing that two members of the TRP receptor family are sensitive to osmotic stimuli, namely TRPV4 (Liedtke 2005) and TRPA1 (Zhang et al. 2008). As mentioned above, both channel proteins appear to be also implicated in nociception in general.

Potassium ions: The most likely explanation for the excitatory action of high concentrations of extracellular potassium ions is a depolarization of the membrane potential due to a reduction of the inside–outside potassium gradient (usually the potassium concentration inside the axon is much higher).

Even at the electron microscopic level, no morphological specializations can be recognized among free nerve endings of different function (mechano-nociceptors, thermo-nociceptors, mechanoreceptors, thermoreceptors). Therefore, the functional differences have been attributed to the presence of special combinations of receptor molecules in the membrane of the endings (Cesare and McNaughton 1997).

2.2.3 Neuropeptide Content of Nociceptors

Most of the following data were obtained in experiments at the spinal level. Trigeminal afferents are not mentioned specifically, but most results are valid also at the trigeminal level.

Whether or not a particular neuropeptide or combination of neuropeptides is associated with a particular functional type of free nerve ending is obscure. Studies on DRG cells indicate that substance P (SP) – and to a lesser extent, also calcitonin gene-related peptide (CGRP) – is present predominantly in nociceptive units (Lawson et al. 1997; Djouhri and Lawson 2004). A strong argument supporting a

nociceptive function of SP is that noxious stimulation in the body periphery is followed by a release of SP in the dorsal horn of the spinal cord and brainstem. On the other hand, there is also evidence speaking against a relation between nociceptive function and the presence of SP. In a study by Leah et al. (1985), ten out of 12 individually identified nociceptive DRG cells of the cat did not exhibit immunoreactivity (IR) for SP. Virtually all afferent units with SP–IR also showed CGRP–IR (Garry et al. 1989); both peptides are presumably released together when the fiber is active. In the spinal cord, CGRP prolongs the action of SP by inhibiting its degradation and by facilitating synaptic transmission in general.

Data from animal experiments in which single DRG cells with receptive endings in muscle were first functionally identified, and then injected with a dye, showed that at least some cell bodies whose peripheral processes terminated in presumable nociceptors contained CGRP (Hoheisel et al. 1994). However, SP, CGRP and other neuropeptides were not only present in nociceptive units but also in other types of muscle receptor (e.g., in some muscle spindles and other low-threshold mechanosensitive (non-nociceptive) units). The only condition for the presence of CGRP seemed to be a small soma size in the DRG and/or a slow conduction velocity of the afferent fiber. Collectively, these data indicate that the correlation between a particular neuropeptide – or a combination of neuropeptides – and the afferent function of the neuron is relatively weak.

Likewise, no neuropeptide has been found that is specific for afferent fibers from a particular tissue. DRG cells projecting in a cutaneous nerve have been reported to contain SP, CGRP, and somatostatin (SOM). The same peptide pattern was found in muscle nerves, whereas visceral afferent units lack SOM (Molander et al. 1987; O'Brien et al. 1989). In comparison to skin nerves, muscle nerves appear to contain less SP. This finding makes sense, because the vasodilatation and plasma extravasation caused by the release of SP and CGRP from free nerve endings (see below) would be dangerous for skeletal muscles, since many of them are surrounded by a tight fascia. Therefore, an SP-induced muscle edema would result in a high increase in interstitial pressure, and could cause muscle necrosis.

On the other hand, there can be no doubt that a relatively high proportion of free nerve endings in muscle exhibit IR to SP and CGRP (Reinert et al. 1998). In a study on functionally identified DRG cells employing a combination of electrophysiological and immunohistochemical techniques, Lawson et al.(1997) reported that cells terminating in cutaneous nociceptive endings showed a strong tendency to express SP, particularly if they had a slow conduction velocity or a small soma in the DRG.

In the CNS, neuropeptides function as so-called neuromodulators, i.e., substances that enhance or attenuate the action of neurotransmitters. Glutamate is generally assumed to be the main neurotransmitter of nociceptive afferents in the spinal cord and trigeminal brainstem, and – with few exceptions – the neuropeptides enhance the central nervous effects of peripheral noxious stimuli (Hökfelt et al. 1980; Kow and Pfaff 1988). The peptides are synthesized in the somas of the DRG or in ganglion cells of cranial nerves. They are transported to both the central and the peripheral terminal of the primary afferent unit. In presynaptic terminals of the

spinal cord and the trigeminal nuclei, neurotransmitters and neuropeptides often coexist, and are released together when action potentials arrive at the terminal.

In a quantitative evaluation of neuropeptide-containing free nerve endings and preterminal axons (both characterized by varicosities) in the GS muscle of the rat, most endings were found around small blood vessels (arterioles or venules), whereas capillaries and the muscle cells proper were not contacted by these endings. Most numerous were the endings containing CGRP followed by SP-positive, VIP (vasoactive intestinal polypeptide) -positive, NGF-positive, and GAP-43 (growth associated protein 43)- positive endings (Reinert et al. 1998). Many endings contained more than one peptide, e.g., SP and CGRP or SP and VIP.

After 12 days of an experimental myositis, the innervation density of the muscle with neuropeptide-containing free nerve endings was significantly increased. The effect was particularly marked for endings containing SP, GAP-43, and NGF (Reinert et al. 1998). The density of the SP-positive fibers doubled in inflamed muscle. Of course, the question arises as to whether this increase was due to inflammation-induced sprouting of the nerve fibers or to an increase in the neuropeptide content of the individual fiber, so that a higher proportion of fibers were above detection threshold in the microscope. The finding that the density of NGF-positive and GAP-43-positive endings increased together with that of an SP-containing one was interpreted as indicating that sprouting had occurred, because NGF and GAP-43 are strongly expressed in growth cones.

2.3 The Nociceptive Afferent Fiber

A nerve fiber consists of an axon [a cylinder of excitable nerve membrane filled with cytoplasm (the axoplasm)] plus a sheath of Schwann cells. Unmyelinated fibers are not naked axons; they are covered by a single layer of Schwann cells. In myelinated fibers, the sheath similarly consists of a single layer of Schwann cells, but here each cell is wrapped around the axon in multiple turns and its cytoplasm squeezed out, so that the membranes of each turn contact each other. This densely packed spiral of Schwann cell membrane is the myelin. In thin myelinated fibers, the myelin consists of a few spiral turns, in thick myelinated ones the number of turns can amount to several dozen. Afferent or sensory fibers conduct action potentials from the periphery to the CNS; their cell body (soma) is located in the dorsal root ganglion (DRG), and the central process of these cells enters the CNS via the dorsal root (in the case of spinal nerves). Some cranial nerves such as the trigeminal, vagus and glossopharyngeal nerve similarly have sensory ganglia that contain the cell bodies of the afferent fibers. The entire neuron from the nerve ending in muscle to the presynaptic terminals in the spinal cord including the cell body in the DRG is a primary afferent neuron. However, there are also unmyelinated afferent fibers in the ventral root: approximately 15% of all ventral root fibers are unmyelinated afferents, the soma of which is situated in the DRG (Applebaum et al. 1976).

Efferent or motor fibers conduct action potentials from the CNS to the periphery; their soma is located in the spinal cord or brainstem and the fibers leave the CNS via the ventral root or cranial nerve motor roots. An exception are postganglionic sympathetic fibers whose cell bodies are mostly located in the sympathetic trunk (e.g., vasomotor fibers that constrict blood vessels).

In the DRG, the correlation between soma diameter and axonal conduction velocity is weaker than generally thought. This correlation was studied in intracellularly stained DRG cells of the cat (Hoheisel and Mense 1987). For group III units (DRG cells having axons conducting at 2.5–30 m/s), there was no significant correlation between soma size and conduction velocity, and for group IV units (with axons conducting at less than 2.5 m/s), the correlation was even negative. Thus, the axonal diameter or conduction velocity of a given group III or IV afferent fiber cannot be inferred from the soma size in the DRG. Usually, the majority of nociceptive free nerve endings will originate from small-diameter DRG cells, but there are also relatively large somata that have nociceptive peripheral endings.

Nociceptive afferent fibers differ from other fibers in that they are equipped with a special type of sodium channel that cannot be blocked by tetrodotoxin (TTX), the toxin of the puffer fish. These channels are called TTX-resistant (TTX-r); they are phylogenetically old, and are characterized by a slow time-course of the sodium currents and long action potential duration (Matsutomi et al. 2006). TTX blocks the conduction of action potentials in nerve fibers that possess TTX-sensitive sodium channels (mostly non-nociceptive small- and large-diameter fibers). Therefore, one of the first symptoms of TTX poisoning is numbness in the affected body region. In contrast, nociceptive fibers are equipped with many TTX-r channels; therefore, TTX has no blocking action on these fibers. Two TTX-r Na^+ channels important for nociception are the voltage-gated sodium channels (Nav) 1.9 and 1.8. Nav 1.9 has been found exclusively in nociceptive primary afferent neurons, whereas Nav 1.8 is present in both nociceptive and non-nociceptive ones (for review, see Djouhri and Lawson 2004). However, the functional distinction between Nav 1.8 and Nav 1.9 may not be as strict as formerly thought, because recently Nav 1.8 has been reported to be involved in neuropathic pain caused by compression or inflammation of DRG cells (Hudmon et al. 2008).

There is evidence indicating that nociceptive fibers from muscle are equipped with TTX-r sodium channels (Steffens et al. 2003). As TTX-resistant Na^+ channels are characteristic for nociceptive fibers, a substance that specifically blocks TTX-r Na^+ channels would be a perfect analgesic.

One of the typical functional properties of a nociceptor is its high mechanical stimulation threshold, i.e., it is not excited by light pressure or muscle movement but requires strong, subjectively painful stimuli for activation. The high mechanical threshold is surprising, considering the fact that a free nerve ending is a fragile structure with a semifluid membrane. The mechanosensitive TRPV4 receptor is being discussed as the main receptor of mechano-nociceptors (Liedtke 2005), but some doubts remain, because the receptor has been described to have a relatively low threshold to the mechanical component of osmotic stimuli (see above).

Possibly, low-threshold receptors can mediate pain sensations under certain circumstances. Recently, a discussion started about how strictly "labeled" the nociceptive endings and central pathways really are (actually, this discussion is very old and never stopped completely). The labeled line theory (or specificity theory) states that there are (high-threshold) nociceptive pathways that elicit pain and non-nociceptive (low-threshold) pathways that mediate nonpainful sensations such as touch and warmth. However, there are clinical and basic research observations indicating that sometimes non-nociceptive stimuli can cause pain in healthy normal subjects (Yarnitsky 2008). One explanation is that the final decision on the nature of a subjective sensation is made by the brain, which uses the *pattern* of the input from the periphery for this decision (and not just the activity in one specialized pathway). At present, the bulk of the available data suggest that in muscle pain research, nociceptive endings and pathways can be distinguished from non-nociceptive ones. Therefore, in this book the distinction between high-threshold nociceptors and low-threshold non-nociceptive endings has been maintained.

2.4 Fiber Composition of a Muscle Nerve

Table 2.1 shows a list of the fiber types that are present in a muscle nerve**.** Two nomenclatures are used: one classifies the nerve fibers after the diameter of the fibers and labels them with the Roman numerals I–IV (Lloyd 1943). This labeling system is generally used for sensory fibers. The other nomenclature uses the conduction velocity as a criterion and labels them with capital Roman letters or a combination of capital Roman letters and Greek letters in lower case (Erlanger and Gasser 1930).

The nerve to a locomotor muscle in the cat (the lateral GS) is composed of approximately one-third myelinated (720) and two-thirds unmyelinated (2,480) fibers (Table 2.2; Mitchell and Schmidt 1983; Stacey 1969). Nearly one quarter of the myelinated (group III) fibers had nociceptive properties in neurophysiological experiments (Mense and Meyer 1985). Of the unmyelinated fibers, 50% are sensory (group IV), and of these, approximately 55% have been found to be nociceptive in the rat (Hoheisel et al. 2005). In the sternomastoid nerve of the rat, the sensory group IV fibers likewise constitute approximately half of all the unmyelinated fibers, and thus account for the great majority of afferent units in that nerve (Sandoz and Zenker 1986).

Data obtained from one muscle nerve cannot be transferred directly to other muscle nerves, because considerable differences exist between different muscles. For instance, neck muscle nerves of the cat contain unusually high numbers of sensory group III receptors (Abrahams et al. 1984). One possible explanation for these differences is that the muscles have different functions and environmental conditions: in contrast to the neck muscles, which must register the orientation of the head in relation to the body in fine detail, the locomotor hindlimb muscles often have to contract with maximal strength and under ischemic conditions. Another

Table 2.1 Fiber types in a muscle nerve

		Type	Function (examples)	Diameter including sheath (μm)	Mean conduction velocity (m/s)
a					
Sensory fibers	Thick myelinated	Group I	Muscle spindle primary (Ia)	15	100
			Golgi tendon organ (Ib)	15	100
		Group II	Muscle spindle secondary	8	50
	Thin myelinated	Group III	Nociceptors	<3	15
			Paciniform corpuscles	<3	15
				<3	15
	Unmyelinated	Group IV	Nociceptors	1	1
			Mechanoreceptors	1	1
b					
Motor fibers	Thick myelinated	Aα	α-Motoneuron	15	100
		Aβ	Skeleto- and fusimotor	8	50
	Thin myelinated	Aγ	Fusimotor to muscle spindle	5	20
		B	Sympathetic preganglionic	<3	15
	Unmyelinated	C	Sympathetic and parasympathetic postganglionic (vasomotor)	1	1

For muscle afferent (sensory) fibers the nomenclature by Lloyd (Group I–IV) has been used (**a**). This system is based on the diameter of the fibers. The other common nomenclature uses Latin and Greek letters and is based on the conduction velocity of the fibers. It is normally applied to efferent (motor) fibers (**b**). For reasons of comparison, the mean conduction velocity is given for both sensory and motor fibers. The two systems are similar in the following respects: Group I and II are thick myelinated and correspond largely to Aα− and Aβ−fibers. Aγ- and Aδ-fibers are thin myelinated and correspond largely to Group III. The unmyelinated C fibers are identical to the Group IV afferents

Table 2.2 Composition of the nerve to the lateral gastrocnemius–soleus muscle in the cat. Notice that the unmyelinated fibers outnumber the myelinated ones by a factor of almost two, and that approximately one-third of all nerve fibers are unmyelinated sensory ones. The majority of the latter are likely be nociceptors (modified after Mitchell and Schmidt 1983)

Fiber numbers (GS nerve)			
Myelinated		Unmyelinated	
Sensory	Motor	Sensory	Motor
480	720	1,000	1,000
Sum myelinated 1,200		Sum unmyelinated 2,000	
Sum all fibers 3,200			

example for the difference between spinal and cranial nerves is that the trigeminal nerve has a lower proportion of unmyelinated fibers.

2.5 Muscle Receptors Other Than Nociceptors

In addition to nociceptors, there are other muscle receptors whose function is essential for the understanding of muscle pain. The most important ones and the nerve fibers supplying them are described below.

Muscle spindles are complex receptive structures that consist of several specialized muscle fibers (the so-called intrafusal muscle fibers; the name is derived from their location inside the spindle-shaped connective tissue sheath. Accordingly, all the "normal" muscle fibers outside the spindle are "extrafusal" fibers). Muscle spindles measure the length and the rate of length changes of the muscle, i.e., their discharge rate increases with increasing muscle length and with increasing velocity of the length change. Their discharge frequency decreases during contraction of the muscle, because they are arranged in parallel to the extrafusal muscle fibers and are relaxed by the contraction. The receptive endings of the spindle form loops around the central portion of the intrafusal muscle fibers. Stretching of that portion is the adequate stimulus for the endings. When the muscle is stretched the loops are deformed, and action potentials are evoked in the fibers connecting the spindle to the central nervous system. These fibers are of three types: the Ia fiber arises from the primary ending that responds to both static length and dynamic length changes and exhibits impulse activity at all muscle lengths. The fiber has a thick myelin sheath and is among the fastest conducting fibers of our body (around 100 m/s; Table 2.1). Group II fibers arise from the secondary ending of the muscle spindle; this is mainly sensitive to length changes (Kandel et al. 2000), and most Group II fibers are not active in a relaxed muscle. In some cases, the secondary endings are supplied by thin myelinated group III fibers. The muscle spindle is the only mechanoreceptor whose sensitivity can be changed by the CNS. To this end, the spindle has motor fibers (Aγ or fusimotor fibers) which cause a contraction of the peripheral parts of the intrafusal fibers and thus stretch the central parts. This "prestretch" increases the sensitivity of the ending to external stretch. The activity of many muscle spindles is used by the CNS to determine the angle of the joint the muscle acts upon. The main central effect of the muscle spindle Ia fiber is excitation of the homonymous muscle (the muscle that harbors the spindle). The Ia fiber has monosynaptic connections with the α-motoneuron; the function of these connections can be tested with one of the tendon jerks, for instance the patellar reflex.

Golgi (tendon) organs measure the tension of the muscle. They are arranged in series with the extrafusal muscle fibers; their location is the transition zone between muscle and tendon. The supplying fiber is the Ib afferent, whose structure is identical to the Ia fiber (thick myelin sheath and high conduction velocity). The receptor has a much simpler structure than the muscle spindle; it consists of receptive endings

that are interwoven between the collagen fiber bundles of the tendon. When the tension of the muscle increases, the endings are compressed and produce an electrical signal. Golgi organs are excited by both muscle contraction and muscle stretch, i.e., during all situations that increase the tension of the muscle. They do not possess a CNS control of their sensitivity. The main central action of the Golgi organ is inhibition of the homonymous muscle. Contrary to the traditional assumption, tendon organs are quite sensitive to stretch: The contraction of a few muscle fibers is sufficient for their activation (Crago et al. 1982). Therefore, the assumption that tendon organs require strong forces for their activation, and prevent the muscle from overload by inhibiting the homonymous α -motoneurons, is questionable.

Muscle spindles and Golgi organs are proprioceptors, i.e., they measure the internal state of the body.

Not all muscles contain muscle spindles and Golgi tendon organs: some of the external muscles of the eye bulb lack spindles and tendon organs (Büttner-Ennever 2007).

Pacinian corpuscles (PC) and paciniform corpuscles. These receptors do not respond to static pressure; they require dynamically changing mechanical stimuli, and are best excited by vibrations of relatively high frequency (close to 300 Hz; Kandel et al. 2000). The receptive ending is formed like a rod, and covered by several concentric membranes which give the receptor an onion-like appearance in cross-sections. Between the membranes, a viscous fluid is present that determines the receptive properties of the ending: a constant pressure stimulus does not excite the receptors, because the fluid moves away and the central rod is under static pressure. Alternating pressure stimuli with a fast onset and offset – such as vibrations – are transmitted to the core of the ending and excite it, because the fluid between the membranes is too viscous to move away quickly. Under these conditions, the receptor behaves like a solid structure that has a rigid connection between the concentric membranes and the receptive ending in the core of the corpuscle.

2.6 Free Nerve Endings in Tendon

The reconstruction of free endings in the calcaneal tendon of the cat at the electron microscopic level yielded various morphological types of free nerve ending connected to group III and IV afferent fibers (Andres et al. 1985). Based on morphological criteria or the location in the tissue, the authors distinguished five types of free nerve ending supplied by group III fibers. One terminated in venous vessels; it had the special feature of a flattened profile in cross-sections, and possessed exposed receptive areas on its edges. Type 2 ended in the wall of lymphatic vessels. Type 3 and 4 supplied the connective tissue around blood vessels, and one of these types had contacts to collagen fiber bundles. The collagen bundles were assumed to transfer mechanical forces to the receptive ending and, therefore, the endings were viewed as mechanoreceptors. The fifth type of group III ending innervated the endoneural connective tissues of small nerve fiber bundles.

In the same study, two types of free nerve endings of group IV fibers were described; both were located in the connective tissue of blood vessels, and some of the fibers contained granulated vesicles. The contents of the vesicles is unknown; they probably contained neuropeptides.

2.7 Free Nerve Endings in Fascia

At present, little information is available about the innervation of fascia. This is an important gap in our knowledge, because fascia is an important component of the musculoskeletal system and likely to contribute to many forms of pain that are subsumed under the label "muscle" pain. One example is low back pain: The thoracolumbar fascia (TF) plays an essential role in body posture and trunk movements (Bogduk and Macintosh 1984). It is not only a passive transmitter of mechanical forces of the low back and abdominal muscles but also contractile by itself (Schleip et al. 2005).

The available data on the innervation of the TF are scarce and partly conflicting. One of the earlier studies even suggested that the TF may not be pain-sensitive (Kuslich et al. 1991). Bednar et al. (1995) studied specimens from the TF of patients with low back pain in the light and electron microscope and found no evidence for specific end organs. They concluded that the TF was deficiently innervated in these patients. One of the reasons for this conclusion was that a few years earlier another group studying human specimens had reported the presence of encapsulated and free nerve endings in the TF (Yahia et al. 1992).

In our group, we have obtained the first – and still preliminary – immunohistochemical data on the innervation of the TF in the rat. In the connective tissue around the superficial lamina of the TF we found many CGRP- and SP-containing free nerve endings. The majority of the fibers were located in the subcutaneous layer, as well as between the fascia and the surface of the multifidus muscle (Fig. 2.8). The SP-positive endings are of particular interest, because they are thought to be nociceptors. The thick collagen fiber bundles of the fascia proper were not richly supplied with free nerve endings. The loose connective tissue around the TF is probably deformed during any trunk movement, and therefore the free nerve endings are strategically situated to sense any disorders in these movements. It is conceivable that overload of the fascia puts mechanical stress and irritation on the endings, and thus may contribute to low back pain.

2.8 Efferent Functions of Nociceptors

2.8.1 Release of Neuropeptides from the Nociceptive Ending

Neuropeptides (e.g., SP, CGRP, VIP, SOM) are stored in vesicles in the varicosities of the peripheral terminal. Whenever a nociceptor is excited, it releases the

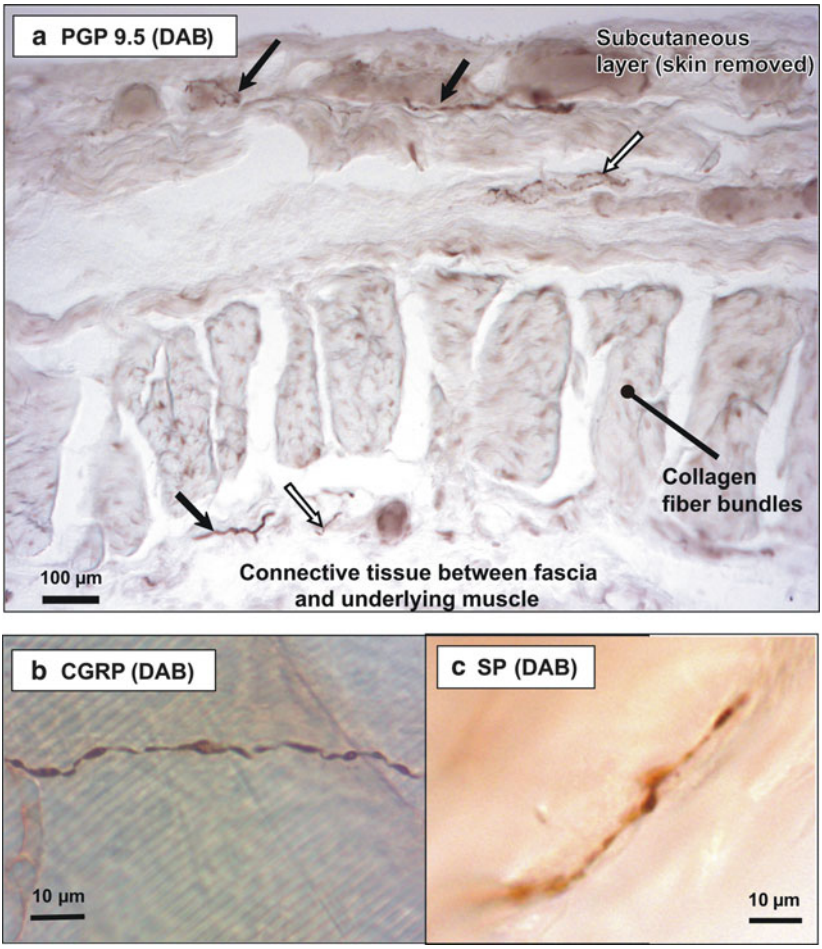

Fig. 2.8 Small-diameter nerve fibers and free nerve endings in the thoracolumbar fascia of the rat. **a** Cross-section in the coronal plane through the fascia at the level of vertebral body L5. The dense collagen fiber bundles that form the fascia proper are cross-sectioned and visible as brick-like structures in the middle layer; on both sides, loose connective tissue connects the collagen bundles to the skin and underlying muscle, respectively. Nerve fibers are mainly present in the loose connective tissue. The receptive free nerve endings are marked by *open arrows* and can be recognized by just visible widenings (varicosities). The *filled arrows* indicate small-diameter fibers of passage. Immunohistochemical fiber staining with antibodies to protein gene product (PGP) 9.5. PGP 9.5 stains all fibers, irrespective of their afferent or efferent nature. The antigen–antibody complex was made visible using the diaminobenzidine (DAB) reaction. **b** Single free nerve ending stained with antibodies to calcitonin gene-related peptide (*CGRP*). CGRP is a neuropeptide that is mainly present in afferent (sensory) nerve fibers, only, but it does not distinguish between nociceptive and non-nociceptive nerve endings. The varicosities are clearly visible. **c** Single nerve ending stained with antibodies to substance P (*SP*). The neuropeptide SP is assumed to be present predominantly in nociceptive fibers

neuropeptides into the interstitial tissue. SP then releases histamine from mast cells, and together with CGRP these agents cause vasodilatation and an increase in vascular permeability of the blood vessels around the active ending. The result is a shift of blood plasma from the intravascular to the interstitial space. Outside the blood vessel, BKN is cleaved from the plasma protein kallidin, serotonin (5-HT) is set free from platelets, and PGs (particularly PGE_2) from endothelial and other tissue cells. All these substances sensitize nociceptors. Thus, the main tissue alteration induced by a nondestructive noxious mechanical stimulus is a localized region of vasodilatation, edema, and sensitized nociceptors.

2.8.2 The Axon Reflex

The release of neuropeptides from an activated nociceptor is an essential aspect of its function. A nociceptor is not a passive sensor of tissue-threatening stimuli; it actively influences the microcirculation and chemical composition of the interstitial space around it. The morphological basis of the axon reflex is that the branches of a single nociceptive ending can extend over a relatively large area (several 100 μm; Fig. 2.9). If a noxious stimulus activates only one part of the ending, the action potentials originating in that region of the ending can invade antidromically (against the normal direction of propagation) those branches of the ending that were not excited by the stimulus. These antidromic action potentials release neuropeptides from the unstimulated branches. The whole process is called the *axon reflex*. It is assumed to be the reason for the visible wheal and flare around a cutaneous lesion. The vascular permeability is increased mainly by SP (and by the neurokinins A and B; Gamse and Saria 1985), whereas CGRP is assumed to act primarily as a vasodilator. There is evidence showing that CGRP enhances the plasma extravasation induced by SP and neurokinins A and B, but reduces the vasodilatory action of SP by desensitizing muscle arterioles to the peptide (Öhlén et al. 1988).

The area of wheal and flare after a localized damage to the skin – for instance around a needle prick – could be an indicator of the extent of the excited nociceptive ending. Of course, this applies only if a single ending is stimulated and if the distance covered by diffusion of the neuropeptides is constant.

The size of the receptive fields (RFs) of cutaneous polymodal nociceptors was found to be less than 2 mm in cat (Bessou and Perl 1969) and 6–32 mm in rabbit (Kenins 1988). A receptive field is that region of the body from which a receptive ending (or a central sensory neuron) can be excited. The above figures are larger than the reported length of the branches of a nociceptor ending (a few hundred μm; Stacey 1969). The difference may be due to the fact that nociceptors can be excited from a certain distance, particularly when coarse probes are used. For a muscle nociceptor, the size of the RF can be determined only with limitations, particularly if it is located deep within the muscle. The reported sizes of superficially located fields or the projections of deep RFs on the muscle surface range from spot-like to

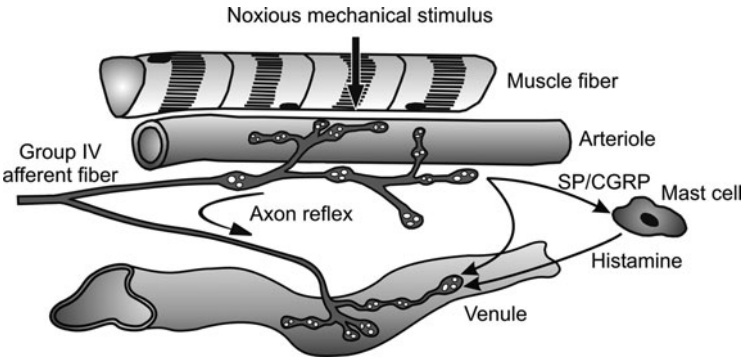

Fig. 2.9 Events occurring around a muscle nociceptor during noxious mechanical stimulation. The nociceptor has two branches, one in the connective tissue around an arteriole, another one close to a venule. The noxious stimulus (*filled straight arrow*) excites the branch on the arteriole; this leads to the release of neuropeptides from the ending such as SP and CGRP. These peptides have a direct action on the small blood vessels in the vicinity of the ending, namely vasodilation and increase in permeability. SP also degranulates mast cells; the released histamine likewise is a vasodilator. Action potentials that are generated in the fiber branch on the arteriole can retrogradely (against the normal direction of propagation) enter the branch on the venule and release neuropeptides. This process is called axon reflex; it is assumed to be responsible for the reddening and swelling of the skin around a localized lesion. The release of neuropeptides from nociceptive endings by retrograde action potentials can also occur in neuropathy or radiculopathy, when action potentials originate in sensory fibers at the site of a nerve compression and are propagated both anterogradely (to the CNS) and retrogradely (to the receptive ending; neurogenic inflammation). The increase in blood vessel permeability by SP is followed by plasma extravasation, which leads to the formation of bradykinin and other agents that sensitize nociceptors. The result of both axon reflex and neurogenic inflammation is a local edema with sensitized nociceptors

several cm^2 in the gastrocnemius muscle of the cat and dog (Kumazawa and Mizumura 1977; Mense and Meyer 1985).

2.8.3 Neurogenic Inflammation

The release of SP, CGRP, neurokinin A, and other agents from nociceptors is the central factor in the cascade of events that lead to neurogenic inflammation in the periphery (Lembeck and Holzer 1979). Neurogenic inflammation is characterized by tissue edema and infiltration by immune cells, i.e., it exhibits the major histological signs of a (sterile) inflammation. It develops whenever action potentials are generated not at the receptive ending but somewhere along the course of primary afferent units (spinal nerve or dorsal root). The action potentials propagate both to the CNS (causing pain) and to the peripheral ending (causing release of neuropeptides and neurogenic inflammation). The published data indicate that vasodilatation can be elicited by antidromic stimulation of both Aδ- and C fibers, but increase in vascular permeability and plasma extravasation by stimulation of C fibers only.

Neuropathies and radiculopathies and other pathological conditions that are associated with antidromic activity in sensory nerve fibers are examples of such events (Marchand et al. 2005). Neurogenic inflammation is likely to increase the dysesthesia and pain of patients suffering from neuropathies.

Neuropeptides also influence immune cells (for a review, see Morley et al. 1987) and synoviocytes. These actions may be of particular importance for the development and maintenance of chronic arthritis and other inflammatory disorders of deep somatic tissues.

Inflammatory disorders are usually accompanied by sensitization of peripheral nociceptors, which is one source of inflammatory pain (for details, see Chap. 3).

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