Effects of changes in pH on the afferent impulse activity of isolated cat muscle spindles

M. Fischer*, S.S. Schäfer

Department of Neurophysiology (Unit 4230), Abteilung Neurophysiologie-OE 4230-Medizinische Hochschule, Hannover Medical School, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

Accepted 23 February 2005
Available online 31 March 2005

Abstract

Muscle spindle activity has been shown to decrease in the sustained contracting muscle. The effect has been assumed to result from a declining fusimotor drive. Since accumulation of metabolites including H⁺, lactate and CO₂ might also affect the receptor in the fatiguing muscle, the impulse activity of muscle spindles isolated from the cat tenuissimus muscle was characterized under varying degrees of extracellular pH, thus excluding any effect on fusimotor activity, blood supply and extrafusal muscle fibers. The isolated receptor was exposed to bathing fluids of pH 6.4, 7.4 and 8.4, and afferent discharge activity was recorded from the spindle nerve. Both primary and secondary endings responded similarly to changes in pH. Resting discharge frequency usually decreased with decreasing pH and increased with increasing pH. A sudden break-off in activity was observed with about 40% of primary endings and about 30% of secondary endings at pH 6.4. Experiments with slow stretch stimulation indicated that this effect was caused by a rising threshold of firing at the encoder site of the endings. With brief ramp-and-hold stretches, we tested the effects of changes in pH on the dynamic and static sensitivity of primary and secondary endings. When pH was reduced from 7.4 to 6.4, the initial burst activity at the beginning of the ramp phase increased in primary and secondary endings and the dynamic response increased in secondary endings, demonstrating that the dynamic properties of muscle spindle endings were usually augmented in the acidic milieu. The static properties rose as well because the static index of both types of ending increased significantly. By contrast, dynamic and static properties of both primary and secondary endings decreased significantly, when pH was increased from 7.4 to 8.4. The amplitude of tension that was measured during the passive stretch stimuli very slightly decreased in the acidic solution and very slightly increased in the alkaline solution. The decrease in the resting discharge activity at low pH supports those previous observations, which demonstrate a reduced peripheral input from muscle spindle afferents to the spinal motor nuclei during fatigue in the isometric contracting muscle. The present finding indicates that an attenuated afferent discharge is not only caused by a decreasing central activation of γ-motorneurons, but may additionally be supported by a direct effect of protons on the muscle receptor itself. The accompanying augmentation of stretch sensitivity is suggested to correspond to the well-known increase in physiological tremor during exhaustive exercise.

© 2005 Elsevier B.V. All rights reserved.

Theme: Sensory systems
Topic: Somatic and visceral afferents

Keywords: Isolated muscle spindle; pH effects; Muscle fatigue; Physiological tremor

1. Introduction

Metabolites including H⁺, lactate and CO₂ accumulate in the cytoplasm of muscle fibers during sustained exercise. Thereby, changes in pH possibly affect a number of cellular functions including enzymatic pathways of cell metabolism, transport mechanisms and ionic currents.
Amino acid side groups of virtually every protein might be protonated during acidification or deprotonated during alkalization, respectively. For that reason, it is important that organisms have developed strategies to keep extracellular and intracellular pH constant. Respiratory and metabolic compensation of acidosis and alkalosis establish a nearly constant pH of 7.4 in the mammalian blood plasma by regulating extracellular pH. In the case of intracellular pH regulation, diverse pumps and carriers transport protons and bicarbonate across the cell membrane, thus efficiently overcoming an intracellular acid load or alkaline load [2,43]. Nevertheless, intracellular pH of muscle fibers decreases to about 6.5 during moderate to about pH 6.7–6.8 during exhaustive exercise [28].

Were also observed in the interstitial space, decreasing to and the venous pH [48]. As a result, low degrees of pH demonstrate that there is a gradient between the interstitial pH and venous pH as being reduced by only a load or alkaline load [2,43]. Nevertheless, intracellular pH regulation, diverse pumps and carriers plasma by regulating extracellular pH. In the case of a nearly constant pH of 7.4 in the mammalian blood metabolic compensation of acidosis and alkalosis establish cellular and intracellular pH constant. Respiratory and that organisms have developed strategies to keep extra- alkalinization, respectively. For that reason, it is important protonated during acidification or deprotonated during 
fibers decreases to about 6.5 during moderate to high intensity exercise [27]. Early studies reveal data for interstitial pH and venous pH as being reduced by only a small degree of about 0.2 units of pH [18,31]. New investigations, however, using microdialysis probes demonstrate that there is a gradient between the interstitial pH and the venous pH [48]. As a result, low degrees of pH were also observed in the interstitial space, decreasing to about pH 6.7–6.8 during exhaustive exercise [28].

This drop in interstitial pH during exercise might influence the activity of muscle receptors and alter their afferent input to the motor nuclei in the spinal cord. Small diameter muscle afferents (groups III and IV) appear to be activated and to reduce motor activity by reflex inhibition of the motoneuron pool [1,17,53]. Muscle spindle afferents (Ia) initially augment activity during isometric muscle contractions as a result of co-activation of γ-motoneurons and α-motoneurons. During sustained voluntary contraction, however, the afferent feedback from muscle spindles to the motoneuron pool has been shown to decrease continuously [35]. It has been suggested that a reduced γ-activation is responsible for that gradually declining peripheral input. Changes in afferent input partly contribute to a reduction of force and EMG (electromyogram) amplitude during sustained maximum voluntary contraction. With submaximal voluntary contraction, however, EMG amplitude increases, resulting from central recruitment of new unfatigued motor units [8,33,34,35]. Concerning muscle spindle activity, Nelson and Hutton [37] observed the following effects when fatiguing the cat gastrocnemius muscle using a 100-Hz tetanic stimulation. An increase in the resting discharge and in the dynamic sensitivity to ramp stretch was observed in primary and secondary endings. The static response, however, was decreased in primary endings and remained unchanged in secondary endings. With both voluntary activation and fatiguing electrical stimulation, changes in fusimotor activity and extrafusal as well as intrafusal muscle fiber stiffness might be involved in the alteration of the afferent response.

The aim of the present study was to investigate the pH effects on the afferent muscle spindle activity excluding γ-activation and possible side effects on blood supply and extrafusal muscle fibers. Thus, an isolated muscle spindle preparation was chosen. Afferent impulses were recorded from the spindle nerve while the bathing fluid of modified pH was exchanged. Using a similar preparation Fukami [16] studied changes in the impulse activity of muscle spindle endings, as affected by NH₃ and CO₂ in order to vary intracellular but not extracellular pH. He observed a decrease in the background activity and in the dynamic and static discharge rates during stretch when intracellular pH of muscle spindle endings was lowered. In the present study, however, dynamic and static sensitivity to stretch increased in the acidic extracellular milieu, even though the resting discharge activity declined.

2. Materials and methods

2.1. Spindle preparation and stretching of the isolated spindle

The tenuissimus muscle and its nerve supply were excised from the hind limb of cats anesthetized with sodium pentobarbital (45 mg/kg i.v.). The nerve–muscle preparation was transferred into a modified Ringer’s solution [40] and fixed in a dissection chamber according to its length in situ when the angle of the femur/tibia joint was adjusted to 135°. Extrafusal muscle fibers and connective tissue were removed from the receptor and its nerve. All branches of the nerve that did not supply the spindle were cut. The isolated muscle spindle was subsequently fixed to holding rods of a stretching device using histoacryl glue (B. Braun-Melsungen). One holding rod was connected to the membrane of a loudspeaker. Movements of that membrane effected stretching of the receptor. The other rod was connected to a miniature mechanoelectrical transducer (SensoNor AE 801) to measure the tension that developed during passive stretch. The transducer consisted of a highly rigid silicon beam (spring constant: 2 N/mm) with two ion-implanted resistors that were connected to a bridge amplifier. A passive tension of 10 mg deflected the beam by approximately 50 nm. Linearity was satisfying within the range of expected spindle tensions (<20 mg, non-linearity: 0.25%). Only the tip of the silicon beam was located in the bathing solution. Neither changes in temperature nor changes in pH affected the transducer. Variations in the perfusion rate of the experimental chamber, however, could slightly shift the tension signal. Thus, the evaluation of tension was restricted to the amplitude of the signal.

Within the experimental chamber, the initial length (L₀) of the muscle spindle was adjusted to correspond to its in situ length, again. The amplitude of ramp-and-hold stretches was usually 5% of L₀. Ramp velocity was 40% of L₀ per second. Stretch was held for 3 s and pauses between repeatedly applied stretches lasted 10–15 s. Slow stretch and slow release experiments were used to determine the critical discharge frequency of muscle spindle endings. In this case ramp velocity was 0.5 or 1% of L₀ per second.
2.2. Recording technique and evaluation of afferent discharge frequencies and tension

The spindle nerve was drawn into an oil-filled chamber and placed on a platinum electrode to provide for extracellular recording of discharges. The reference electrode was placed close to the isolated spindle in the bathing solution. Usually, the activity of one to three muscle spindle endings was recorded simultaneously. Impulses of different endings were distinguished by their different amplitudes. Differentiation between primary and secondary endings was achieved by various physiological criteria that have been described previously [12]. One of the most reliable criteria of differentiation was the property of primary endings but not secondary endings to discharge with each cycle of a very small (5 μm) sinusoidal stretch in the frequency range of 10–100 Hz.

The responses of individual endings to ramp-and-hold stretches were evaluated by determining their instantaneous discharge frequencies from the sequence of action potentials. Responses to at least five stretch stimuli were superimposed in a diagram to build up a discharge pattern as displayed in Fig. 1 for a primary muscle spindle ending. Certain basic discharge frequencies were extracted from these discharge patterns. IA is the initial discharge frequency displaying the resting activity of an ending. IA is calculated as the median value of the instantaneous discharge frequencies during the last 500 ms prior to stretch. With primary endings an initial burst of activity usually occurs at the start of a stretch and forms the initial peak (IP) in the discharge pattern. The maximum discharge frequency during that initial burst determines the IP value. Secondary endings seldom produce an initial burst, but often show a steep initial increase in the firing rate at the start of a stretch. The IP value of secondary endings was therefore determined as the maximum discharge frequency of the steep initial increase. The peak dynamic discharge frequency (PD) is reached at the end of the ramp phase of the stretch. PD is calculated as the median value of the last 25 ms of the ramp phase. Two static values were obtained from the plateau phase of the stretch. The one is the maximum static value (MST) that is calculated as median value over a period of 50 ms, usually starting 20 ms after the beginning of the plateau phase following the early fast adaptive decay. The other is the final static value (FST) that is calculated as the median frequency of the last 250 ms of the plateau phase, following the slow adaptive decay of firing. The adaptive decay from PD to FST is defined as the dynamic response (DR). The difference between FST and IA displays the static index (SI).

Three characteristic values were obtained from averaged tension curves (see Fig. 9a). The resting tension (IT) prior to stretch, the peak tension (PT) at the end of the ramp phase and the final static tension (ST) at the end of the plateau phase were determined.

Significance of pH effects was analyzed by using the paired Student’s t test.

2.3. Ringer’s solution and exchange of bathing fluids

The ionic composition of the Ringer’s solution was: 118.6 mM NaCl; 4.75 mM KCl; 1.80 mM CaCl2; 23.2 mM NaHCO3; 1.19 mM KH2PO4; 0.84 mM MgSO4; 2.40 mM glutamine; 3.20 mM glycine; 0.97 mM histidine; 1.02 mM glutamic acid; 1 g/l glucose [40]. The solution was aerated with 95% O2 and 5% CO2. The temperature was kept constant at 35°C. Just before an experiment started, the pH was adjusted to 6.4, 6.9, 7.4, 7.9 and 8.4 by an application of appropriate amounts of HCl or NaOH, respectively. Aerating the Ringer’s solution was stopped after pH adjustment to avoid changes in pH resulting from further application of CO2. With non-aerated solutions, we observed a very small drift in pH of less than 0.05 degrees within 30 min. During the experiments, however, acidic and alkaline solutions were usually applied to the muscle spindles over a period of 5 min only. Further control experiments denied the risk of hypoxia since non-aerated Ringer’s solution (pH 7.4) did not influence muscle spindle activity over a period of at least 30 min.

The experimental chamber containing the isolated muscle spindle had a volume of 1 ml and was continuously perfused with the Ringer’s solution. Using a perfusion rate of 3–5 ml/min, the bathing fluid was exchanged within a period of at the most 20 s. Changes in pH were achieved by switching the source of Ringer’s supply using solutions of differing pH.
3. Results

3.1. pH effects on the resting impulse activity of muscle spindles

The effects of changes in pH were studied on 32 isolated muscle spindles. Results were obtained from 24 primary endings and 30 secondary endings. Fig. 2 shows a preliminary experiment without application of stretch stimuli, where changes in the resting impulse activity of a primary ending (Ia, upper panel) and a secondary ending (II, lower panel) of one isolated muscle spindle were investigated under step-like variations of pH. The Ringer’s solution of pH 7.4 was replaced by Ringer’s solutions of pH 6.4, 6.9, 7.9 and 8.4, respectively. The endings’ responses to the rising and falling pH were superimposed in each panel. The graphs beneath each panel depict the changes in pH that occurred in the bath.

The experiment exemplifies one of the main pH effects that has been shown to be statistically significant during further investigation: the resting discharge frequency increases with increasing pH and falls with decreasing pH (see also pH effects on the initial activity in Fig. 5). The upper trace of each panel displays the response of the muscle spindle endings when pH was increased from 7.4 to 8.4. With both types of ending, the resting impulse frequency increased. The effect was stronger for the secondary ending (increase by about 16 imp/s) than for the primary ending (increase by about 7 imp/s). When pH was raised from 7.4 to 7.9, there was a transient increase in the impulse frequency of the primary ending when the bathing solution was exchanged. The secondary ending reached an enhanced firing rate that kept constant over the whole period of altered pH. The effects of acidification were stronger on the primary ending than on the secondary ending. The discharge activity of the primary ending suddenly fell silent, when the pH of the bathing solution was reduced to 6.4. This again is a typical pH effect for many muscle spindle endings. The abrupt stop in firing was not to be expected since the impulse frequency seemed to have just reached a steady state in the acidic solution. When the bathing solution was replaced by a Ringer’s solution of pH 7.4 again, it took some minutes for that primary ending to recover (not shown in this figure). Effects of acidification on the resting impulse activity of the secondary ending were small in comparison with the effects of alkalinization (lower traces in panel II). A decreasing pH from 7.4 to both 6.9 and 6.4 reduced the resting discharge activity by approximately 5 imp/s.

In the pool of 32 isolated spindles, we generally observed reversible changes in resting activity of both types of ending, and the time to recover was usually short. However, the degree of changes markedly varied from one ending to the other. Acidification often produced weaker effects than alkalinization. The transient fluctuations in activity, that were repeatedly observed with respect to changes in pH, were not due to experimental artifacts being produced by the exchange of the bathing fluid, since such fluctuations had never been observed in control experiments, when solutions of identical pH were exchanged. Thus, these fluctuations might result from counteracting pH effects, possibly influencing the muscle spindle at different sites. In order to examine the underlying mechanisms in more detail, we studied the muscle spindle responses under ramp-and-hold stretches. In this way, it should be possible to separate in a first approach those effects that are stretch-dependent from those effects that are not stretch-dependent.

3.2. pH effects on muscle spindle activity during ramp-and-hold stretches

Fig. 3 shows three representative examples of the variable pH effects on the four basic discharge frequencies that characterize the endings’ response to a ramp-and-hold stretch. The initial activity (IA), the peak dynamic discharge value (PD) at the end of the ramp phase of the stretch, the maximum static discharge value (MST) at the beginning of the hold phase and the final static discharge value (FST) at the end of the hold phase of the stretch are plotted against time. Fig. 3a shows data from a primary ending and Figs. 3b
and c show data from secondary endings that belong to different muscle spindles. The spindles were repeatedly stimulated by 4–5 stretches within 1 min. Horizontal bars at the top of each diagram represent the periods where the pH of the bathing solution was changed from 7.4 to 8.4 and 6.4, respectively. Thin vertical lines in panel (c) mark a pause in data acquisition. IA of the primary ending remained nearly unaffected, but IA of the secondary endings increased with alkalinization and decreased with acidification. The difference between PD and IA is decreased at pH 8.4 for the endings in panels (a) and (c), and increased for each ending at pH 6.4. The secondary ending in panel (c) ceased firing in the acidic milieu. IA and – with a certain delay – PD, MST and FST dropped to zero at pH 6.4. The effects of changes in pH were generally reversible.

Even though pH effects appear to be inconsistent at a first glance, they probably result from very few basic processes that are superimposed and build up the different pH-dependent reactions. It may be helpful to introduce these basic processes before focusing on the examples of Fig. 3. The first pH effect is a shift in the firing rates that is independent of stretch. We believe that this process affects all the basic discharge frequencies in common. Thus, we define it as a shift in the basic level of activity. The second process is a pH-dependent change in stretch sensitivity. This process influences the stretch-dependent discharge values PD, MST and FST but not IA that represents the stretch-independent resting activity. Accordingly, effects on PD, MST and FST reflect the sum of changes in (1) the basic level of activity and (2) the stretch sensitivity, but effects on IA exclusively reflect changes in the basic level of activity.
After all, the response of an ending depends on the strength of each single pH effect. However, the strength varies from one ending to the other, as will be shown for some examples.

With the primary ending in Fig. 3a, the initial activity IA remained nearly unaffected by changes in pH. Only a small decrease may be observed with a decreasing pH from 8.4 to 7.4. However, stretch-dependent discharge frequencies markedly changed with varying pH. PD declined from about 65 to 50 imp/s when pH increased from 7.4 to 8.4, and it inclined from about 65 to 85 imp/s when pH decreased from 7.4 to 6.4. The basic discharge frequencies MST and FST were influenced in a similar way as PD was, but the effects were less. The responses of that primary ending exemplify a very weak pH effect on the basic level of activity, but a very strong effect on the stretch sensitivity. Stretch sensitivity decreases with increasing pH and rises with decreasing pH.

Fig. 3b shows pH effects on a secondary ending. Here, a strong effect on the level of activity and a very weak effect on stretch sensitivity is exemplified. IA was markedly increased from 45 to 58 imp/s when pH was increased to 8.4. Simultaneously, PD inclined from 61 to 72 imp/s. MST and FST increased by nearly the same amount as PD did. With acidification, all the basic discharge frequencies transiently fell and thereafter slightly increased to reach a steady state value that was reduced for IA (48 to 42 imp/s) but very slightly changed for PD, MST, and FST. Along these lines, an elevated pH tends to shift the basic level of activity to higher discharge frequencies and a decreasing pH tends to shift the level of activity to lower discharge frequencies.

Fig. 3c displays two experiments with another secondary ending. The thin vertical lines separate the first from the second registration. A pause of about 30 min lay between the two registrations. With this secondary ending, the two basic effects of pH forcefully influenced the afferent discharge pattern in common: an increase in pH from 7.4 to 8.4 induced an increase in IA from 36 imp/s to 46 imp/s. FST increased from 43 imp/s to 50 imp/s, but MST and PD remained nearly unaffected. This is consistent with an increase in the basic level of activity that is superimposed by a reduction in the stretch sensitivity. The shift in IA indicates the increase in the level of activity. MST and PD remain constant because the activating shift is balanced by an inhibition effect when stretch sensitivity is reduced.

When the bathing solution of pH 7.4 was exchanged by a solution of pH 6.4, IA declined from 38 imp/s to about 30 imp/s. PD, MST, and FST also transiently decreased, indicating that the basic level of activity was reduced. According to a pH-dependent increase in stretch sensitivity, the values of PD, MST, and FST inclined after that transient reduction. When the discharge values had nearly reached a steady state, however, IA was the first that suddenly dropped to zero. Similarly, the discharge frequencies PD, MST, and FST abruptly dropped to zero. This drop occurred with a delay of 1–2 min as compared to the break-off in IA. Thus, stretch-dependent impulse activity continued for a while before the ending completely fell silent in the acidic environment. When the bathing solution was replaced by normal Ringer’s solution (pH 7.4) the secondary ending recovered within 4–5 min. PD, MST, and FST re-appeared at first. Two minutes later, IA occurred as well. The sudden inactivation at pH 6.4 occurred with about 40% of primary endings as well as with about 30% of secondary endings. The phenomenon represents a third basic component of pH effects.

In order to generalize the findings, we calculated the mean values of the four basic discharge frequencies from the data of numerous primary and secondary endings and plotted these values against pH (Fig. 4). For this evaluation, it was essential to separate those endings that fell silent at pH 6.4 from those endings that did not. With four primary endings MST, FST and IA but not PD dropped to zero. Even though PD remained, we decided to handle these endings as if their impulse activity entirely broke off. However, a solely drop of IA to zero was not assessed as being a break-off in activity since it is known that many muscle spindle endings usually do not discharge at rest. The following criterions were used to sort the muscle spindle afferents into two groups for each type of ending.

Group Ia-1 (10 primary endings; Fig. 4a) and group II-1 (21 secondary endings; Fig. 4c) consist of those endings that form a PD, MST, and FST in each solution of varying pH. The value of IA is not taken into account.

Group Ia-2 (7 primary endings; Fig. 4b) and group II-2 (8 secondary endings; Fig. 4d) consist of those endings that form a PD, MST, and FST in bathing solutions of pH 7.4 and 8.4. Additionally, MST, FST, and IA drop to zero at pH 6.4. PD may or may not be developed at pH 6.4. It is noteworthy that PD dropped to zero at pH 6.4 for all of the secondary ending, but not for all of the primary endings.

Endings that did not fit to one of these groups were excluded from evaluation. Thus, seven out of 24 primary endings did not fit because they did not develop a FST at pH 7.4 and 8.4, respectively. With secondary endings, only one out of 30 endings did not fit because this ending was active at pH 8.4 only.

Fig. 4 shows the mean values and standard deviations of the four basic discharge frequencies of primary endings and secondary endings. For the sake of greater clarity, the symbols for MST and FST were plotted with a slight horizontal displacement in each diagram. Results that were obtained from group Ia-1 primary endings are presented in Fig. 4a. The mean value of IA clearly rose with increasing pH from 13 imp/s at pH 6.4 to 33 imp/s at pH 8.4. This corresponds to the finding that indeed seven out of 10 endings showed a pH-dependent increase in their initial activity and only one ending’s IA decreased. The IA value of two primary endings of the group was almost not affected by changes in pH. However, these two endings did respond to changes in pH with changes in PD, MST and FST.
Effects of pH on the basic discharge frequencies PD, MST, FST were highly variable in the group Ia-1. When pH was raised to 8.4, the values of PD, MST and FST sometimes increased collectively (similarly to the secondary ending’s responses in Fig. 3b), and sometimes they fell collectively (similarly to the responses in Fig. 3a). Moreover, with some afferents PD decreased, while MST and FST increased. On average, the variable results abolished the pH effects in the diagram of Fig. 4a. However, the variability that has been observed is plausible when it holds true that increasing pH increases the basic level of activity on the one hand and decreases stretch sensitivity on the other. Since the values of PD, MST and FST depend on both pH effects, the net outcome will depend on the strength of each of the two effects. If the effect on the level of activity dominates, then PD, MST and FST will rise and fall in parallel with IA. If, however, the effect on stretch sensitivity dominates, then PD, MST and FST might even fall with increasing pH when IA (displaying the level of activity) simultaneously increases. The declining distance between the curves of PD and IA in Fig. 4a indicates that on average the stretch sensitivity of primary endings fell with increasing pH.

Fig. 4b shows the results of group Ia-2 primary endings. Mean values of MST, FST and IA were 0 imp/s at pH 6.4 according to the activity break-off in the acidic solution that was a criterion of the group. Mean PD was 43 imp/s at pH 6.4 resulting from the finding that four out of seven primary endings still developed a PD and three did not. With inclining pH from 7.4 to 8.4 mean values of PD and MST slightly fell, the mean value of FST remained constant and the mean value of IA slightly increased. The difference between PD and IA decreased. Again, the results are compatible with an increasing basic level of activity and a decreasing sensitivity to stretch when pH is increased from 7.4 to 8.4.

Fig. 4c displays the mean values and standard deviations of the basic discharge frequencies obtained from group II-1 secondary endings. The mean value of IA increased from 23 imp/s at pH 6.4 to 43 imp/s at pH 8.4. The basic discharge frequencies PD, MST and FST were less affected. The mean value of PD remained constant at about 67 imp/s, MST slightly inclined from 57 to 60 imp/s and FST inclined from 46 to 54 imp/s. The general finding is similar to that obtained with primary endings: Secondary endings reduce their sensitivity to stretch when pH is increased in the bathing solution because the distance between the curves of PD and IA decreases. The effects for secondary endings are even stronger than for primary endings. The same holds true for secondary endings of the group II-2 (Fig. 4d). Each of
the basic discharge frequency is 0 imp/s at pH 6.4. When raising the pH from 7.4 to 8.4 the four basic discharge frequencies rise in concert, that is, the facilitating pH effect on the basic level of activity dominates over the depressing effect on stretch sensitivity. The fact that the increase in IA is stronger than the increase in PD indicates that stretch sensitivity is again slightly diminished (PD-IA decreases).

Are pH effects on the basic level of activity significant? In order to answer that question, we compared the values of IA under varying pH, as IA is the only discharge value that is not influenced by additional pH effects on stretch sensitivity. Fig. 5 shows the mean values and standard deviations of IA obtained from 11 primary endings (Ia) and 24 secondary endings (II). The evaluation was restricted to those endings that developed an IA at pH 7.4. Mean values are presented for pH 7.4 (dark bars), pH 6.4 (hatched bars) and pH 8.4 (light bars). With seven out of 11 primary endings IA dropped to zero in the acidic solution and the mean value of IA fell from 28 imp/s to 12 imp/s. By contrast, IA increased in the alkaline solution reaching a mean value of 33 imp/s. With 11 out of 24 secondary endings, IA dropped to zero at pH 6.4. Mean IA fell from 32 imp/s to 20 imp/s. Alkalization increased mean IA to 44 imp/s. For both types of ending the pH effects on IA were significant.

Moreover, we tested the significance of pH effects on the sensitivity to stretch. Results are shown in Figs. 6a and b for primary endings and in Figs. 6c and d for secondary endings. The diagrams show two different components of the stretch-dependent response of muscle spindle afferents: (1) the dynamic response DR is the sum of two adaptive decays that occur during the hold phase of the stretch. It is composed of the fast adaptive decay from PD to MST that is affected by the velocity of stretch, and the slow adaptive decay from MST to FST that is influenced by the amplitude of stretch [5,24,41]. Therefore, IP is a further discharge pattern of secondary endings. However, we often observed it in the discharge patterns of most primary endings, but rarely in the discharge patterns of secondary endings. Nevertheless, IP was well developed in the discharge pattern of secondary endings at the beginning of a stretch that shares the physiological characteristics of the initial burst in primary endings [14]. Thus, we evaluated the initial increase of secondary endings in the same way as the initial burst of primary endings and used the same abbreviation (IP) for the sake of simplicity.

3.3. Effects of changes in pH on the initial burst

An initial burst of firing often occurs in muscle spindle endings at the beginning of a stretch and forms an initial peak (IP) in the discharge pattern (Fig. 1). The initial burst has been attributed to a short-range elasticity in intrafusal muscle fibers, and its peak discharge frequency has been shown to strongly depend on the ramp velocity of a stretch [5,24,41]. Therefore, IP is a further discharge frequency that represents dynamic properties of muscle spindle endings. IP was well developed in the discharge patterns of most primary endings, but rarely in the discharge patterns of secondary endings. However, we often observed a steep initial increase in the discharge frequency of secondary endings at the beginning of the stretch that shares the physiological characteristics of the initial burst in primary endings [14]. Thus, we evaluated the initial increase of secondary endings in the same way as the initial burst of primary endings and used the same abbreviation (IP) for the sake of simplicity.

We calculated the amplitude of the initial peak and initial increase as the difference IP-IA, with the intention of subtracting any pH effect on the level of activity from the pH effect on the dynamic properties that determine IP. Sixteen primary endings developed an initial burst at the beginning of a stretch. However, only four out of these 16
endings generated an IA at each degree of pH. With these four primary endings, the mean value of IP-IA increased from 57 imp/s to 66 imp/s, when the bathing solution was acidified from pH 7.4 to 6.4. The mean value of IP-IA decreased to 37 imp/s, when pH increased to 8.4. Even though the pH effect was rather strong, the number of endings appeared to be too small to generalize the effect. With the purpose of achieving a sufficient number of samples, we decided to include the endings, where IA dropped to zero at low pH. Notably, subtracting IA from IP could in that case not successfully eliminate changes in the level of activity. Therefore, the difference IP-IA might be erroneously small for the acidic milieu, where a reduction of the basic activity might continue to decrease IP, while IA is fixed at 0 imp/s. A facilitating pH effect on IP-IA might thus be covered by a strong pH-dependent decrease in the level of activity. Nevertheless, Fig. 7(a) shows for the collective of 16 primary endings that the facilitating effect of acidification on IP was still strong enough to increase the mean value of IP-IA from 68 imp/s at pH 7.4 (dark bar) to 72 imp/s at pH 6.4 (hatched bar). With alkalization mean IP-IA fell significantly to 56 imp/s (light bar).

With secondary endings (II in Fig. 7), we observed similar pH effects. A steep initial increase was detected in the discharge patterns of 14 secondary endings. On average, IP-IA increased from 32 imp/s to 41 imp/s when pH decreased from 7.4 to 6.4. A decrease to 19 imp/s was observed, when pH rose from 7.4 to 8.4. The effects were significant for both directions of change in pH. In the case of secondary endings, the effects remained even significant, when the evaluation was restricted to those eight endings that produced an IA at each degree of pH.
We like to append an observation on three primary endings that did not contribute data to the last evaluation because they built up an extraordinary large IP-IA value of about 200–350 imp/s at pH 7.4 (IP-IA was usually less than 100 imp/s). IP-IA of two out of these three endings even grew with an increasing pH. This finding indicates that the amplitude of IP might be regulated by more than one pH-dependent mechanism, as will be debated in the discussion.

3.4. Effect of pH on the critical discharge frequency

Cessation of activity was observed in the acidic solution for both primary and secondary endings. About 40% of primary endings and about 30% of secondary endings fell silent when the isolated muscle spindle was exposed to pH 6.4. Since usually IA dropped to zero at first and PD, MST and FST were affected with a certain delay, we tried to examine whether a shift in the critical discharge frequency ($f_{\text{crit}}$) of an ending might explain the cessation of firing. From previous investigations, it is known that muscle spindle afferents and other mechanoreceptors stop firing when the discharge rate falls below a certain threshold [11, 19]. This threshold varies from one individual ending to the other. We tried to measure the threshold of firing for several endings by slowly reducing the length of the isolated muscle spindle until those afferents fell silent that were active at the initial length $L_0$. The lowest rate of firing was defined as being the critical discharge frequency of the individual ending. The velocity of the slow ramp relaxation and the slow ramp stretch was within a range of 0.5 and 1% of $L_0$ per second.

In experiments on six afferents (two primary endings and four secondary endings), we were successful in measuring the critical discharge frequency. With these endings, we studied the time course of changes in the critical discharge frequency during acidification and recovery from acidification, respectively. Fig. 8a represents an example for the instantaneous discharge frequencies of a primary ending under successive slow stretch and release stimuli in a solution of pH 6.4, just before the ending completely stopped firing. Changes in spindle length are depicted in the graph underneath (ramp velocity 1% $L_0$/s). The lowest discharge rate during each stretch was determined and marked by $f_1$–$f_3$. The increases in the discharge values $f_1$–$f_3$ from one stretch to the other represent the time course of changes in the critical discharge frequency of the ending. (b) Changes in the critical discharge frequency ($f_{\text{crit}}$) of individual endings (thin lines) and mean changes in $f_{\text{crit}}$ (thick lines) are plotted against time. Curves are synchronized on the left hand side by the moment of complete cessation of firing in the acidic solution of pH 6.4 ($f_{\text{crit}}$, $t_1$) and on the right hand side by the moment of first re-activation during recovery from complete inactivity in the solution of pH 7.4 ($f_{\text{crit}}$, $t_2$). Broken lines display the minimum $f_{\text{crit}}$ that could be accurately measured for an individual ending. The responses of individual endings as well as the mean response show a steep increase in $f_{\text{crit}}$ before firing abruptly stopped. Changes in $f_{\text{crit}}$ are reversed during recovery.
solution of pH 6.4. The graph underneath depicts the length changes of the isolated spindle. The figure shows the period in time where the critical discharge frequency dramatically increased from one test stimulus to the other. The lowest discharge frequency (= critical discharge frequency) that occurred during the slow ramp phase of each stretch stimulus was marked by $f_1$, $f_2$ and $f_3$, respectively. The value of the critical discharge frequency increased from $f_1 = 24$ imp/s during the first stretch to $f_2 = 30$ imp/s during the second stretch and to $f_3 = 36$ imp/s during the third stretch. With the next stretch stimulus (not shown) firing completely stopped.

Fig. 8b displays the time course of changes in the critical discharge frequency for some individual endings (thin lines) and the mean effect (thick line). The resolution in time is limited by the interval between succeeding measurements, and therefore is given by the period of repeatedly applied slow stretches (about 20 s per stimulus cycle). The individual curves on the left hand side were synchronized by the moment of complete cessation of firing in the acidic solution of pH 6.4 (first vertical line, $t_1$), and curves on the right hand side were synchronized by the moment of first re-activation during recovery from complete inactivation (second vertical line, $t_2$). The endings’ activity recovered in a solution of pH 7.4. Broken lines display the minimum critical discharge frequencies that could be accurately quantified. As soon as spontaneous firing occurred assessment of the critical discharge frequency was impossible. For explanation, see the uppermost curve on the right hand side. With recovery from inactivation, we were able to measure the exact values of the critical discharge frequency during four subsequent stretch stimuli. During the first stretch, we obtained a value of 43 imp/s, during the fourth stretch, we measured 38 imp/s. During the fifth stretch and the following stretch stimuli, the firing did not anymore fell below the critical discharge frequency of the ending because the ending developed a high frequency spontaneous discharge. Therefore, we do not know the exact value of the critical discharge frequency in that period, but we know that the value was less than 38 imp/s (broken line). It appears plausible to assume that the critical discharge frequency should decrease further. Obviously, the spontaneous discharge frequency of this particular ending was extraordinarily high compared to the spontaneous discharge frequencies of the other endings. For that reason, we decided to exclude the data of that individual ending from the calculation of the average curve (thick line).

The responses of individual endings as well as the mean response clearly show a steep increase in the critical discharge frequency before firing abruptly stopped. On average, the critical discharge frequency rose from 19 imp/s to 33 imp/s within about 3 min before the endings fell silent in the acidic solution. A further increase in the critical discharge frequency is certainly needed for the complete cessation of firing in experiments with enhanced muscle spindle activity under brief ramp-and-hold stretches (e.g., Fig. 3c). When the acidic bathing solution was replaced by Ringer’s solution of pH 7.4 again, a clear decrease in the critical discharge frequency occurred during recovery. The time course and amplitude of that response reversed the effect that was observed during acidification.

3.5. Effects of changes in pH on the tension of isolated muscle spindles

Changes in stiffness of intrafusal muscle fibers affect the tension of the whole isolated muscle spindle when it is passively stretched [14]. The tension of isolated muscle spindles was investigated under varying pH using stretch amplitudes of 5% of $L_0$. The velocity of stretch was 40% of $L_0$ per second. Tension curves were averaged from responses to 5–10 subsequent stretch stimuli. Three basic values were obtained from each tension curve (Fig. 9a), that
is, the initial tension IT prior to the start of a stretch, the peak tension PT at the end of the ramp-phase of the stretch and the final static tension ST at the end of the hold-phase of the stretch. Fig. 9a exemplifies two averaged tension curves of one muscle spindle. A thin line represents the tension that was recorded during a stretch at pH 7.4. A slightly thicker line represents the tension that was observed during a stretch at pH 8.4. The change in length is depicted by the graph underneath. The amplitude of passive tension was increased when pH was raised from 7.4 to 8.4. The tension at the end of the ramp phase (PT) was a little more augmented than the tension at the end of the hold phase of the stretch (ST). Unfortunately, the entire tension signal was slightly shifted by small fluctuations in the perfusion rate of Ringer’s solution passing through the experimental chamber that irritated the very sensitive mechanoelectrical transducer. Thus, we focused our attention on changes in the amplitude of the tension curves. Subtracting IT from PT and ST respectively eliminated from consideration the misleading shifts in tension.

The bar chart of Fig. 9b shows the mean results and standard deviations derived from seven isolated muscle spindles. The pairs of bars on the left hand side represent the changes in PT-IT and ST-IT when the bathing solution of pH 7.4 (dark bars) was exchanged by a solution of pH 6.4 (hatched bars). The pairs of bars on the right hand side represent the changes in PT-IT and ST-IT when the bathing solution of pH 7.4 (dark bars) was exchanged by a solution of pH 8.4 (light bars). Note that the reference bars (pH 7.4) differ slightly in magnitude because they were obtained from different control experiments just prior to changes in pH. On average, the peak tension amplitude (PT-IT) fell by 6% from 3.73 mg to 3.50 mg, when the bathing solution was acidified from pH 7.4 to 7.4. The static tension amplitude (ST-IT) fell by 4% from 3.21 mg to 3.08 mg. Although the effects were small, the changes during acidification were significant using the paired Student’s t test (P < 0.05). When the pH of the bathing solution was raised from 7.4 to 8.4, PT-IT grew insignificantly from 3.45 mg to 3.69 mg and ST-IT grew insignificantly from 3.01 mg to 3.11 mg.

What the figures do not show is that the effects on tension and the effects on the firing rate, as reported in the previous sections, did not strictly correlate in time. Sometimes, the effects on tension followed the promptly starting changes in the firing rate with a certain delay. This observation indicates that pH effects on impulse activity are probably not the result of pH-dependent changes in muscle spindle tension.

4. Discussion

With an increase in extracellular pH from 6.4 to 7.4 and to 8.4, the main effects on isolated cat muscle spindles were as follows: (1) the resting activity of both types of muscle spindle ending increased. (2) The dynamic and static sensitivity to stretch decreased with both types of muscle spindle ending. (3) The initial peak discharge frequency of the endings decreased. (4) The tension obtained during passive stretch of the spindle slightly increased. (5) 30–40% of the muscle spindle endings abruptly ceased firing when being exposed to an acidic solution of pH 6.4.

By using an isolated receptor preparation, the findings are not influenced by side effects of acidosis and alkalosis on extrafusal muscle fibers and blood supply that might both be able to indirectly affect the afferent impulse activity. However, there are a lot of sites within the receptor that might be influenced by varying concentrations of protons including the sensory membrane and encoder membrane of the afferent nerve fibers, their axons as well as the intrafusal muscle fibers and the spindle capsule. It is additionally worth notifying that extracellular pH regulation is generally excluded with the isolated receptor preparation, but intracellular pH regulation might be present. Intracellular acidification and alkalinization possibly activated compensatory transport mechanisms that might be responsible for the transient increases and decreases in the afferent discharge frequency as frequently observed when the pH of the bathing solution was changed. The transient fluctuations in the firing rate of the primary ending at pH 7.9 in Fig. 2 (curve b of the upper panel) may give an example for the outcome of those regulatory processes. Other examples may be the early transient changes in the basic discharge frequencies of the secondary endings in Figs. 3b and c, where the muscle spindle was exposed to a bathing solution of pH 6.4.

The following sections of the discussion will focus on a few main topics. (1) Does a change in extracellular pH affect the intracellular pH of muscle spindle endings? (2) Do changes in extracellular pH affect the mechanical properties of the muscle spindle? (3) What are the effects of varying pH on the electrical properties of the sensory ending membrane and encoder membrane? (4) What are the functional implications of the present results?

4.1. Effects of changes in extracellular pH on the intracellular pH

To our knowledge, a single previous report by Fukami [16] described pH effects on isolated cat muscle spindles. The results of that report and the findings of the present study agree in some but not all aspects. Fukami investigated the background activity, the dynamic peak frequency and the static discharge rate that resemble the basic discharge frequencies IA, PD and FST of our study. Fukami used solutions containing either NH₄Cl or CO₂ to alter intracellular pH while keeping the extracellular pH constant, that is, a well known method for loading a cell with H⁺ or removing H⁺ from a cell’s interior, respectively [43,49]. Unfortunately, sensory endings of muscle spindles are too small to be impaled by microelectrodes for a direct measurement of changes in intracellular pH. Thus, Fukami...
speculated that a decrease in the background activity and in the dynamic and static discharge rates, as being observed in the NH₄Cl containing solution, was affected by intracellular acidification of the muscle spindle endings. An intense increase in the dynamic peak frequency and a gradual increase in the static discharge rate and background activity appeared to be correlated with intracellular alkalization.

Comparing these results with the findings of the present investigation, the declining IA with extracellular acidification is in agreement with the declining background activity as observed by Fukami under intracellular acidification. Concerning stretch-dependent discharge rates, we interpret changes in the values PD and FST as the sum of at least two pH effects. The one influences the basic level of activity that is decreased with acidification, and the other influences the stretch sensitivity that is increased with acidification. Therefore, we observed variable shifts in PD and FST depending on the strength of each of these counteracting effects. When the effect on stretch sensitivity was rather weak and the effect on the basic discharge activity was rather strong, then we observed a decrease in PD and FST with extracellular acidification that is similar to the observations of Fukami concerning intracellular acidification. However, when the effect on stretch sensitivity dominated, then PD and FST increased with extracellular acidification (Fig. 3a).

Fukami did not analyze stretch sensitivity. Fig. 1 of his report, however, shows that the dynamic peak frequency is more strongly reduced with intracellular acidification than background activity. Therefore, stretch sensitivity may be considered to decline in his experiments. We observed an increase in stretch sensitivity with extracellular acidification. Fig. 2 of Fukami’s report shows that the dynamic peak frequency is more strongly augmented than the background activity with intracellular alkalization. Therefore, stretch sensitivity may be considered to incline in his experiments. We observed a decrease in stretch sensitivity with intracellular alkalization.

The discrepancy in these details may result from the different experimental arrangements. With the experiments of Fukami changes in pH affected exclusively intracellular targets, since extracellular pH was kept constant. With our experiments, however, intra- and extracellular effects have to be considered. Previous investigations show that extracellular acidosis is expected to decrease intracellular pH, by directly inhibiting acid-extruding transporters like the Na-H exchanger [2,3,43]. Additionally, extracellular acidosis enhances the bicarbonate efflux via a chloride-bicarbonate exchanger that will leave H⁺ behind in the intracellular space. Both processes lower intracellular pH. Thus a decrease in the extracellular pH is usually accompanied by a decrease in the intracellular pH. Likewise, an increase in the extracellular pH is accompanied by an increase in the intracellular pH. It seems to be essential, however, that the induced change in the intracellular pH is usually smaller than the change in the extracellular pH [4,47]. Thus, extracellular acidification, as induced in our experiments, will produce a declining proton gradient from the extracellular to the intracellular side. With intracellular acidification in Fukami’s experiments, however, the proton gradient declines in the opposite direction from the intracellular to the extracellular space. Accordingly, it is possible to hypothesize that the pH effect on stretch sensitivity depends on the direction of the proton gradient across the cell membrane of sensory endings. In that case, the results of both investigations agree very well.

4.2. Effects of pH on the mechanical properties of isolated muscle spindles

Changes in tension were measured under passive stretch of the isolated muscle spindles. We observed a small increase in the amplitude of passive tension when extracellular pH was increased. A small decrease in tension was observed when extracellular pH was decreased (Fig. 9). These changes in tension directly reflect changes in the viscoelastic properties of the receptor. It is known from skinned fiber preparations that reducing pH decreases stiffness as well as active tension in cardiac and skeletal muscle fibers [36,38,46]. The effects result from a decrease in the number of attached cross bridges and a reduction of the single cross bridge force. An accumulation of bridges in the low force state of the cross bridge cycle is suggested [46]. Additionally, protons may directly lower the Ca²⁺ affinity of Troponin C that might additionally reduce tension [38].

According to these findings, a decrease in the passive tension of intrafusal muscle fibers is to be expected, when extracellular and, in particular, intracellular pH decreases. Our results confirm the expectations and may reflect an interaction of protons with the contractile proteins of intrafusal muscle fibers. However, the changes in amplitude of the passive tension were quite small. The miniature effects might be explained by at least two reasons. (1) The contribution of intrafusal muscle fibers to the tension of the whole receptor is relatively small. Previous findings demonstrate that the major part of passive tension is supplied by the capsule of the receptor and by residual connective tissue surrounding the capsule [14]. Thus, the changes in tension that occur in that tissue may easily occlude the minor changes induced by a change in stiffness of intrafusal muscle fibers. (2) pH effects on intrafusal muscle fibers depend on changes in their internal pH. However, changes in intracellular pH are usually less than the experimentally provoked changes in extracellular pH [4,47]. We do not know the ratio of intracellular to extracellular changes in pH. If the ratio is small and large alterations in extracellular pH induce only minor alterations in intracellular pH, then the minute effects on tension would not be surprising.

With a decreasing number of attached cross bridges and a reduced force per cross bridge, it might be expected that the
initial peak in the afferents’ discharge patterns should decrease when pH is lowered, since it is generally agreed that the initial peak is related to cross bridges that are formed between actin and myosin filaments in the poles of the resting intrafusal muscle fibers [5,9,24,39,41,42,45]. However, we did not measure that diminution in IP in the present study. On the contrary, we observed an increase in IP-IA with both types of ending, when pH was lowered from 7.4 to 6.4. Moreover, a decrease in IP-IA was shown to be significant when pH was increased from 7.4 to 8.4 (Fig. 7). The problem may be solved, when changes in pH affect the electrical properties of the muscle spindles more strongly than their mechanical properties. Strong effects on the electrical properties might easily overcome the tiny effects on the mechanical properties that were observed in our experiments. Thus, the expected decrease in the initial peak under acidification might be more than compensated by an increase in the dynamic sensitivity of the sensory endings (see Section 4.3). Therefore, pH effects on tension are probably not responsible for most of the pH-dependent changes in the afferent activity. In a few exceptional cases, however, effects on mechanical properties appeared to dominate. With two primary endings that developed an extraordinary large IP, demonstrating a strong dependence of firing upon mechanical properties, we really observed a decrease in IP-IA when extracellular pH was reduced, as has been proposed when low pH efficiently reduces stiffness.

4.3. Effects of pH on the electrical properties of isolated muscle spindles

The main effects of pH on muscle spindle afferents were similar for both primary and secondary endings. With extracellular acidification, we observed a reduction of the resting activity (Fig. 2; I A in Fig. 5), indicating a decrease in the basic level of activity, and we observed an increase in the dynamic sensitivity (DR in Fig. 6; IP-IA in Fig. 7) and static sensitivity to stretch (SI in Fig. 6). Reverse effects occurred with extracellular alkalization.

According to the surface potential theory, the excitability of membranes in general might be reduced under enlarged extracellular concentrations of H\(^+\) or divalent cations [15,22]. The theory implies that H\(^+\) and divalent cations bind to negatively charged sites at the external surface of the membrane and thus increase the gradient of the electric field across the membrane. The effect mimics a hyperpolarization of the membrane. The decrease in the basic level of activity of muscle spindle endings that occurred under an increased extracellular H\(^+\) concentration (low pH) might be related to that reduction of membrane excitability. A similar reduction of activity was observed when the calcium concentration of the bathing solution was increased from 1.8 to 3.6 mM [13]. However, increasing external calcium additionally reduced the muscle spindle’s sensitivity to stretch. By contrast, increasing the concentration of H\(^+\) potentiated stretch sensitivity. Therefore, pH effects on stretch sensitivity might rely on a specific interaction of protons with certain ion channels of the sensory membrane.

Unfortunately, there is little knowledge about the ion channel composition of the sensory membrane and the encoder membrane of muscle spindle endings. Stretch-activated channels (SA channels) and voltage-gated calcium and potassium channels and calcium-dependent potassium channels have been considered to be involved in the generation of the receptor potential of muscle spindles [14,25,26,32]. But effects of changes in pH have not been tested for these channels of the spindle. Studies on other ion channels often show a reduced permeability at low pH [7,10,20,22,35,51,52], but pK\(_a\) values for titratable side groups of the channel proteins were typically far away from physiological pH (axonal sodium and potassium channels: pK\(_a\) = 4.4–5.8; SA channels: pK\(_a\) = 9.0). With so little knowledge about pH effects on each of the various depolarizing and hyperpolarizing currents in muscle spindle endings, there is a wide field for speculation. Blocking of the calcium-dependent potassium channel, for instance, that is thought to accelerate spindle adaptation [32] might increase the stretch response in the acidic environment.

Nevertheless, there is one finding that appears to be definitely related to a specific pH effect at the encoder site of the sensory endings. It is the increase in the critical discharge frequency that causes cessation of firing at pH 6.4 (Fig. 8). A blockade of impulse conductance along the axon is certainly not responsible for that activity break-off. With an axonal blockade of action potentials, the impulse activity during the stretch stimuli (PD, MST and FST) should cease simultaneously with the resting activity (IA) in the pauses between individual stretches. By contrast, our results show that low frequency firing is restrained at first, where the sensory membrane and encoder membrane are only slightly depolarized. Thus, resting activity ceases, but PD, MST and FST remain nearly unchanged. The large depolarization of the membrane, as evoked during stretch, is obviously still super-threshold. Finally, a substantial increase in the firing threshold suppresses also high frequency discharge during the stretch, even though the sensory membrane is certainly as strongly depolarized as before.

4.4. Functional implications

Macefield et al. [35] reported that force production and the amplitude of the electromyogram (EMG) decline during prolonged isometric contractions in man. Various mechanisms have been suggested to modulate the neural drive to the muscle during fatigue including motoneuron adaptation [29,30], recurrent inhibition [33], a decline in the excitatory input from muscle spindle afferents [35], reflex inhibition mediated by feedback from group III and IV muscle afferents [1,17,53], and a decline in long loop reflex activity [8]. Enhancing the descending drive from supraspinal centers may compensate for the loss of excitation from peripheral afferents on motoneurons [33–35].
The present experiments support the hypothesis that the peripheral excitatory input to the motoneuron pool is reduced during sustained isometric muscle contraction. Macefield et al. [35] proposed that fatigue of the fusimotor support reduces afferent spindle activity. The authors suggested that fusimotor activity fails to overcome unloading of the spindle in the sustained contracting muscle. We propose a supplementary mechanism: With our experimental arrangement, any effect on γ-motoneurons is ruled out. We believe that H⁺ accumulation in the fatigue muscle directly affects the sensory endings of the muscle spindle because we observed a declining level of activity for the isolated spindle when pH was reduced. Thus, excitatory input to the motoneuron pool might be additionally reduced by a pH-dependent depression of activity at the receptor site.

Moreover, it has been shown that recovery from fatigue is prolonged when the muscle’s blood supply is occluded, also indicating that the reduction of motoneuron drive to the fatigue muscle is supported by peripheral mechanisms in ischemic muscles [1,17,53]. Accumulating metabolites were thought to activate group III and IV afferents of the muscle, resulting in reflex inhibition of the motoneuron pool. With accordance to the findings of the present study, we suppose that occlusion of the blood supply slows down the removal of H⁺ in the fatiguing muscle. The resulting decrease in pH reduces the basic level of activity in muscle spindle afferents. Thus, we hypothesize that a decreasing activity of group Ia and II afferents from muscle spindles is an additional factor of the reduced peripheral drive to the motoneuron pool under ischemic conditions.

The other main finding of the present study was an increase in stretch sensitivity of muscle spindles in the acidic environment. This pH effect may contribute to the well-known enhancement of physiological tremor that is to be observed during a sustained fatiguing contraction [21,34,54]. Hagbarth and Young [21] recognized that stretch reflex activity contributes to the enhanced tremor during exercise since EMG bursts in the fatigue muscle follow volleys of muscle spindle discharges with a constant delay of about 20 ms that corresponds to the length of the reflex pathway. Cresswell and Löscher [6] also suggested that tremor during fatigue partially relies upon peripheral feedback from large fiber afferents because tremor amplitude decreased, when the afferent input to the motoneuron pool was experimentally reduced. We suppose that an increase in stretch sensitivity of muscle spindle endings, as documented for low pH values in the present study, is ideally suited to augment the stretch reflex response under fatigue. The increased dynamic sensitivity, as is evident from the increase in IP and DR at low pH, may in particular contribute to an oscillatory feedback that might promote tremor.

In conclusion, even though the present results are consistent with previous physiological observations, different details concerning the mechanisms of the pH effects are unknown and need further investigation. It would in particular help to clarify the underlying mechanisms when getting further knowledge of the composition and pH sensitivity of ion channels in the sensory membrane and encoder membrane of muscle spindle endings.

Acknowledgment

We wish to acknowledge gratefully the technical assistance of Birgit Begemann during the experiments and the evaluation of data.

References
