Modulation of Action Potential Trains in Rabbit Saphenous Nerve Unmyelinated Fibers

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Abstract

Usually, the main axon is assumed to faithfully conduct action potentials (APs). Recent data have indicated that neural processing can occur along the axonal path. However, the patterns and mechanisms of temporal coding are not clear. In the present study, single fiber recording was used to analyze activity-dependent modulation of AP trains in the main axons of C fibers in the rabbit saphenous nerve. Trains of 5 superthreshold electrical pulses at interstimulus intervals of 20 or 50 ms were applied to the nerve trunk for 200 s. The interspike intervals (ISIs) for these trains were compared to the input interstimulus intervals. Three basic types of C fibers were observed in response to repeated stimuli: first, the ISI between the first and second AP (ISI\textsubscript{1–2}) of type 1 was longer than the interstimulus interval; second, the ISI\textsubscript{1–2} of type 2 showed wavelike fluctuations around the interstimulus interval, and third, the ISI\textsubscript{1–2} of type 3 exhibited shorter intervals for a long period. Furthermore, both 4-aminopyridine-sensitive potassium and hyperpolarization-activated cation currents were involved in the modulation of ISI\textsubscript{1–2} of train pulses. These data provide new evidence that multiple modes of neural conduction can occur along the main axons of C fibers.

Key Words

Single fiber recording · C fiber · Interstimulus interval · Interspike interval · Conduction velocity · 4-Aminopyridine · ZD7288

Introduction

Classically, the role of axons has been limited to the simple propagation of action potentials (APs). The pattern of firing is determined by a number of specialized regions that are responsible for signal integration, such as receptors, the axon hillock, and the synapse. Once initiated, APs propagate regeneratively and distribute uniformly over axonal arbors. Therefore, the axon is usually considered as faithfully carrying signals over long distances and working like a relatively simple relay line \cite{1, 2}. However, recent studies on rat and human axons have challenged this classic view by showing that patterns of spike generation can be modulated as the APs travel down the axons \cite{3–7}. Changes in axonal AP propagation during repetitive stimulation depend on the aftereffects of the preceding APs, that is, that immediately after the conduction of one AP, the axon undergoes a series of
changes in oscillatory excitability, and this has been termed the recovery cycle [8]. In addition, the sequence of changes in axonal excitability after AP firing has been analyzed in detail [5, 9–12]. The recovery cycle can last for several hundreds of milliseconds, which gives rise to periods of enhanced and reduced axonal excitability. Concurrent speeding and slowing of conduction can be observed in the same C fiber in response to a train of twin-pulse stimuli [5, 13]. In previous work conducted by our laboratory, the activity-dependent effect on conduction velocity (CV) was influenced by the frequency and time course of the repeated stimulus as well as the period between conditioning and test pulses [14]. These results suggest that the activity-dependent conduction properties possess non-linear characteristics, which may give rise to complex firing behaviors.

Weidner et al. [5] reported that trains of two and four superthreshold electrical stimuli at varying repetition rates could result in temporal dispersion or narrowing of the interspike interval (ISI) of train impulses. It was concluded that at a given degree of neural accommodation, all afferent C-units exhibit a uniform pattern of aftereffects, independent of fiber class [5]. However, in our recent study of AP firing in the saphenous nerve, when electrical stimulation at different frequencies was used to test the effects of activity-dependent changes in CV, a novel fluctuation of ISIs and three distinct types of conduction failure were observed in the propagation course along C fibers [15]. This result strongly suggests that the effects of previous pulse activity (i.e. aftereffects) on ISIs may have a multiple, nonlinear dynamic courses. Thus, we propose that the ISI in saphenous C fibers might be subjected to distinct patterns of aftereffects, which would exhibit various temporal firing patterns.

In order to verify this hypothesis, regular trains containing 5 pulses for a total of 200 trains were used to repetitively stimulate the saphenous nerve, and changes in the properties of the ISIs in the main axon of single C fibers were measured and analyzed. Three types of C fibers were distinguished according to the distinct patterns of ISI changes, especially the ISI1–2 (the ISI between the first and second AP). Both 4-aminopyridine (4-AP)-sensitive potassium current (I4-AP) and hyperpolarization-activated cation current (ICa) were involved in the regulation of ISIs. These results confirmed, at least in part, the existence of temporal pattern processing along the main axon of C fibers; we termed this phenomenon conduction coding.

Materials and Methods

Animals and Surgery

Adult rabbits of both sexes, weighing between 1.8 and 2.2 kg, were used under a research protocol approved by the Fourth Military Medical University. Anesthesia was induced with sodium pentobarbital (40 mg/kg i.v.). The procedure for animal surgery has been published in our previous study [15]. As shown in figure 1a, the saphenous nerve of one hind leg was exposed at three sites where skin pools were prepared. One site was used for electrical stimulation (S), one site for single fiber recording (R), and another site was used for drug application (D).

Electrophysiological Recording of Single C Fiber Discharges in vivo

Electrophysiological recordings of single C fibers from saphenous nerve were made as previously described [15]. Briefly, in the stimulation pool, a pair of platinum stimulating electrodes was placed under the nerve trunk and covered with warm (about 37°C) mineral oil. In the middle drug application pool, the nerve trunk was separated about 4–5 cm. After carefully removing the epiperineurium, this part of the saphenous nerve was covered with warm physiological saline with or without drug. In the recording pool, a fine filament was teased from the saphenous nerve and the proximal site was placed on a pair of platinum electrodes (30 µm in diameter) for single C fiber recording (fig. 1a). APs were amplified and recorded on a computer with a signal sample rate of 10 or 100 KHz. The CV of a fiber was determined by dividing the distance between the stimulating and recording electrodes by the AP latency. Fibers with a CV of <2 m/s were classified as C fibers [16, 17].

Electrical Stimulation

After a single fiber had been identified, the whole nerve trunk was stimulated electrically (pulse duration 0.8 ms). In the present experiment, a standard repeated stimulus with an intensity 50% greater than the threshold was applied and 5 pulses for a total of 200 trains were delivered (1 train / s) (fig. 1b). The interstimulus interval of the quintuplet pulses was selected for 20, 30, and 50 ms according to the experimental requirement. After starting the stimulation protocol, we waited for 10 min between two stimulation series to allow recovery from the effects of each stimulus train.

The initial CV (CVi) was calculated from the distance between the stimulating and recording electrodes divided by the latency of the first AP. This CVi was compared with the CV of each AP in response to repetitive stimulus pulses at different intervals, and the ratio, as a percentage of CVi, was called CV slowing.

Parameters Used to Evaluate the Temporal Coding and Conduction Properties of C Fibers

To investigate the temporal coding changes during stimulation, we calculated the ISI between the first and second AP (ISI1–2) in every quintuplet pulse. The other three ISIs (i.e. ISI2–3, ISI3–4, and ISI4–5) were assessed in the same way. The difference between the interstimulus interval and ISI was named ΔISI. The difference between ISI1–2 and the interstimulus interval was termed ΔISI1–2. The other three ΔISIs (i.e. ΔISI2–3, ΔISI3–4, and ΔISI4–5) were assessed in an analogous manner. If two consecutive APs had the same CV, their ISIs should exactly match the interstimulus interval, that is, ΔISI = 0. If the ISI was longer than the...
interstimulus interval (i.e. the CV of posterior AP was slower than the preceding one), ∆ISI was defined as positive, whereas ‘faster’ CV for posterior AP leading to shorter ISI was defined as negative ∆ISI.

To determine whether conduction properties of C fibers changed across fibers, we analyzed the rCV according to the methods previously published [13]. In response to the quintuplet electrical pulses, 5 APs were initiated. For reasons of clarity, we named the first AP the leading AP and the other four APs the trailing APs (AP1–4). To investigate the activity-dependent slowing of CV, we determined how the CV of the leading AP changed from the first to the last quintuplet pulse. To exclude the difference among C fibers, we normalized the data by calculating the rCV of the leading AP for each quintuplet pulse, which is denoted as the CV of the leading AP for a given quintuplet pulse divided by the CV of the leading AP for the first quintuplet pulse. We named this rCV for the leading AP with the symbol $L_{n} = \frac{CV_{L,n}}{CV_{L,1}} \times 100\%$, where CV$_{L,n}$ is the CV of the leading AP for the $n$th quintuplet pulse, and CV$_{L,1}$ is the CV of the leading AP of the first quintuplet pulse. An $L < 100\%$ indicates a slowing of CV. To investigate whether the CV of each AP initiated by the quintuplet pulses changed during the whole stimulation, we computed the rCV of each AP by normalizing the data. For a given quintuplet pulse, the CVs of the trailing APs were divided by the CV of the leading AP.
preceding AP, which is designated by the symbol \( T \). \( T_{i,n} = (CV_{1,n}/CV_{1,n-1}) \times 100\% \), where \( T_{i,n} \) is the rCV of the first trailing AP for the \( n \)th quintuplet pulse and \( CV_{1,n} \) is the CV of the first trailing AP of the \( n \)th quintuplet pulse. \( CV_{1,n-1} \) is the CV of the first trailing AP of the \((n-1)\)th quintuplet pulse. The other three \( T_i \)s (i.e., \( T_2 \), \( T_3 \), and \( T_4 \)) were assessed in an analogous manner. \( T >100\% \) indicates that the posterior trailing AP has a faster CV than the preceding one.

Statistical Analysis
All data are represented as means ± SEM. Data were analyzed using a paired or unpaired Student’s t test as appropriate. \( p < 0.05 \) was considered statistically significant.

Results

Classification of C Fibers
In the present study, electrophysiological experiments were performed on 30 rabbits. The mean CV of C fibers was 0.91 ± 0.01 m/s (n = 40). A total of 200 quintuplets of superthreshold electrical pulses at interstimulus intervals of 50 ms were repetitively applied to the trunk of the saphenous nerve at a frequency of 1 Hz. Following the electrical stimulus, we measured the ISI between two successive APs evoked by the same interstimulus interval. Three Types of C Fibers
To investigate the activity-dependent changes in the conduction properties of the three types of C fibers, we compared and analyzed the degree of the activity-dependent slowing (fig. 3). All three types of fibers exhibited activity-dependent slowing of CV where the velocity of the last leading AP was always less than the CV of the first leading AP. However, the conduction properties of the trailing APs were distinguished from the lead APs in these three types of C fibers. For type 1 fibers, the CV of the trailing APs was always slower than the leading AP (fig. 3a1, a2). For type 2 fibers, the CV of the trailing APs was initially slower, then faster, and then again slower than the CV of the leading AP at the end of the trial (fig. 3b1, b2). For type 3 fibers, the CV of the trailing APs was initially slower for a short time and then became faster than the CV of the leading AP for a long period (fig. 3c1, c2). However, there was no obvious difference among the CVs of the last trailing APs (i.e. trailing AP2, trailing AP3, and trailing AP4). In addition, as shown in figure 2, the other three ISIs (\( ISI_{1-3} \), \( ISI_{1-4} \), and \( ISI_{1-5} \)) did not show any significant difference, but nearly matched the inter-
Fig. 2. Three types of ISI change and firing patterns induced by the quintuplet stimuli with the interval of 50 ms. a1 A segment of original recording corresponding to MaxΔISI1–2 from a type 1 C fiber (in a2), which shows a bigger ISI1–2. a2 The ISI series alteration in this type 1 C fiber during the 200 trains. Each point represents an ISI of two APs. Note that the ISI between the first and second AP (ISI1–2) was always bigger than the interstimulus interval. a3 Firing pattern of the initial two APs of the unit in a2. Top: trace of the initial two APs evoked by the electrical quintuplet pulse at 50 ms. For each of the successive traces, the response latencies of the initial two APs are tracked by solid lines from top to bottom. The difference between ISIs and interstimulus intervals is denoted as ΔISI. b1 A segment of original recording corresponding to the point of MaxΔISI1–2 from a type 2 C fiber (in b2), which shows a small ISI1–2. b2 The ISI series alteration of this type 2 C fiber during the 200 trains. Note that the ISI1–2 exhibited a wavelike alteration compared to the interstimulus interval. b3 Firing pattern of the initial two APs for the unit shown in b2. The difference between ISIs and interstimulus intervals is denoted as ΔISI. c1 A segment of original recording corresponding to the point of MaxΔISI1–2 from a type 3 C fiber (in c2), which shows a markedly small ISI1–2. c2 The ISI series alteration of this type 3 C fiber during the 200 trains. Note that the ISI1–2 was bigger than the interstimulus interval at the beginning, then smaller than 50 ms and remained small thereafter. c3 Firing pattern of the initial two APs of the unit shown in c2.
stimulus interval. Therefore, in the following text, we focused on the analysis of changes in conduction properties of the first and second APs.

A scatter plot of the normalized CV of the leading AP (L, see details in Methods for normalization procedure) and the rCV of the trailing APs (T, see Methods) for each fiber is shown in figure 3a3, b3, and c3. Three parameters were used to characterize the activity-dependent changes in CV. As a measure of ‘activity-dependent slowing/speeding’, we computed L for the 200th train pulses (L_{200}).
To quantify the possible supernormal conduction that might exist or develop during the trial, we measured the rCV of the last trailing AP1 (i.e. $T_{1,200}$) and the largest degree of slowing/speeding of the first trailing AP among all the 200 trains (Max $T_1$). As depicted in figure 3a3, both $L_{200}$ and $T_{1,200}$ of type 1 C fibers were <100% during the stimulation train, suggesting a CV slowing of the leading AP and the trailing AP1. For type 2 C fibers, $L_{200}$ was <100% but $T_{1,200}$ experienced a wavelike change, showing less, more, and finally less than 100% under the whole stimulus (fig. 3b3). This is consistent with the CV change of the leading AP and the trailing AP1 in figure 3b2. For type 3 C fibers, $L_{200}$ exhibited <100% but $T_{1,200}$ displayed >100% (fig. 3c3), which indicates that the CV slowing of the leading AP and CV speeding of the trailing AP1 occur in response to the same stimulus.

To further assess whether these three types of C fibers have distinct conduction populations, we compared the changes in $ISI_{1-2}$ and rCV of the leading AP and the trailing AP1 among the three types of C fibers. To sum up, a scatter plot of Max $ISI_{1-2}$ versus $ISI_{1-2}$ of the last quintuplets of firing ($ISI_{1-2,200}$) is shown in figure 4a. The type 1 fiber cluster has both positive $ISI_{1-2,200}$ and Max $ISI_{1-2}$. The cluster of type 2 has negative $ISI_{1-2,200}$ but positive Max $ISI_{1-2}$. The cluster of type 3 has negative $ISI_{1-2,200}$ and Max $ISI_{1-2}$. b Scatter plots of the $T_{1,200}$ versus Max $T_1$. Three populations are apparent; they are separated by a vertical line drawn at 100% and a horizontal line drawn at 100%. The cluster of type 1 has $T_{1,200}$ and Max $T_1$ of <100%. The cluster of type 2 has $T_{1,200}$ of >100% and Max $T_1$ >100%. The cluster of type 3 has $T_{1,200}$ and Max $T_1$ >100%. c Relationship between rCV of the last leading AP ($L_{200}$) and CV1. It was shown that these three types of C fibers have different populations of CV1: type 1 (closed circle, $n = 13$); type 2 (open circle, $n = 12$), and type 3 (star, $n = 15$).
was AP. For type 2 fibers, the rCV of the trailing AP 1 under-
of type 1 fibers was always slower than that of the leading AP. For type 2 fibers, the rCV of the trailing AP underwent an oscillatory change. MaxT 1 was >100%, but T 1,200 was <100%. In contrast, both T 1,200 and MaxT 1 of type 3 fibers were >100%. Taken together, these results suggest that the huge change in ISI 1–2 might be associated with a CV slowing of the leading AP and a CV speeding/slowing of the trailing AP 1 for these three types of C fibers.

The Stimulus Frequency Dependence of ISI Change of Type 3 C Fibers

In figure 5, typical response patterns of type 3 C fibers after the 200 quintuplets of stimuli are compared at different interstimulus intervals (20, 30, and 50 ms). The scatter plot of ISIs showed that the ISI 1–2 exhibited a pronounced increase compared to the other ISIs (ISI 2–3, ISI 3–4, and ISI 4–5) in response to the same stimulus (fig. 5a–c). To measure the change in the ISI 1–2 at different stimulus frequencies, two parameters were designated, MaxΔISI 1–2 and ΔISI 1–2EI (fig. 5c), where MaxΔISI 1–2 indicates the maximal difference between ISI 1–2 and the interstimulus interval, and ΔISI 1–2EI represents the interval when ISI 1–2 reached an entrainment level [5]. As indicated in figure 5g, the average MaxΔISI 1–2 significantly increased from 4.15 ± 0.48 ms at an interstimulus interval of 50 ms to 7.57 ± 0.38 ms at an interval of 30 ms and 11.35 ± 0.34 ms at an interval of 20 ms (p < 0.05 compared to the interval of 50 ms, n = 10). The average ΔISI 1–2EI also significantly increased from 2.48 ± 0.47 ms for an interstimulus interval of 50 ms to 6.24 ± 0.35 ms for an interval of 30 ms and 9.65 ± 0.48 ms for an interval of 20 ms (p < 0.05, n = 10). The histogram shows that both MaxΔISI 1–2 and ΔISI 1–2EI increased in a stimulus frequency-dependent manner during the 200 trains.

To determine whether changes in the average CV of each AP is related to different interstimulus intervals for the 200 quintuplets, three parameters were determined: rCV of the leading AP (L), rCV of the trailing APs (T 1, T 2, T 3, and T 4), and maximal rCV of the trailing AP 1 for each train (MaxT 1 ). As indicated in figure 5d–f, an increase in stimulus frequency led to more activity-dependent slowing of the leading AP (L <100%), and the trailing AP displayed an enhanced CV speeding (T 1, T 2, T 3, and T 4). The rCV of the leading AP (L 200) increased from 105.14 ± 0.62% for 50 ms to 108.60 ± 0.61% for 30 ms and 110.65 ± 0.96% for 20 ms (p < 0.05 compared with the interstimulus interval of 50 ms, n = 10). The average T 1,200 was enhanced from 103.10 ± 0.44% for 50 ms to 106.55 ± 0.52% for 30 ms and 108.50 ± 0.95% for 20 ms. In comparison, the average L 200 decreased from 78.90 ± 1.11% for 50 ms, 74.55 ± 0.85% for 30 ms, and 69.73 ± 0.76% for 20 ms. Taken together, these results suggest that the degree of change for ISI 1–2 and activity-dependent conduction properties of type 3 C fibers might be associated with the stimulus frequency.

**ISI Change Reduced by 4-AP**

In our previous study, a fluctuation in ISIs was observed before conduction failure and 4-AP-sensitive potassium current (I 4,AP) was involved in the regulation of conduction failure patterns. To further investigate whether I 4,AP mediates changes in ISIs and CV of each AP initiated by the electrical stimulus, we examined the effects of 4-AP on ISI changes and rCV in response to the quintuplet pulses for a total of 300 trains at the interstimulus interval of 20 ms in type 3 C fibers. As shown in figure 6a–c, ISI 1–2 became obviously closer to the interstimulus interval with the increase in 4-AP concentration (20 and 40 μM). The average MaxΔISI 1–2 was significantly reduced from 12.75 ± 0.51 ms for control C fibers to 10.52

ΔISI 1–2, 200, while the type 3 fiber cluster exhibited a negative MaxΔISI 1–2 and a negative ΔISI 1–2, 200. Similarly, we found that there are three populations for the rCV of the trailing AP and the leading AP. The normalized data is shown in figure 4b, c. Both T 1,200 and MaxT 1 of type 1 fibers were <100%, suggesting the CV of the trailing AP 1 of type 1 fibers was always slower than that of the leading AP. For type 2 fibers, the rCV of the trailing AP underwent an oscillatory change. MaxT 1 was >100%, but T 1,200 was <100%. In contrast, both T 1,200 and MaxT 1 of type 3 fibers were >100%. Taken together, these results suggest that the huge change in ISI 1–2 might be associated with a CV slowing of the leading AP and a CV speeding/slowing of the trailing AP 1 for these three types of C fibers.
ZD7288 promoted changes in ISI with an increase in concentration (10–20 μM) for type 3 C fibers. More importantly, changes in MaxΔISI$_{1-2}$ and ΔISI$_{1-2}$EI were larger at higher concentrations of ZD7288. As indicated in figure 7g, the average MaxΔISI$_{1-2}$ significantly increased from 12.01 ± 0.40 ms for control C fibers to 14.50 ± 0.58 ms for 10 μM ZD7288 and 17.05 ± 0.59 ms for 20 μM ZD7288 (p < 0.05 compared to control C fibers, n = 8). The average ΔISI$_{1-2}$EI also significantly increased from 8.77 ± 0.70 ms for control C fibers to 9.51 ± 0.43 ms for 10 μM ZD7288 and 12.11 ± 0.56 ms for 20 μM ZD7288 (p < 0.05, n = 8). In contrast, L$_{300}$ decreased during the stimulus train, showing activity-dependent CV slowing of the leading AP after the application of ZD7288. Additionally, both T$_{1, 300}$ and MaxT$_{1}$ increased during the stimulus, exhibiting activity-dependent CV speeding of the trailing AP at the end of the trial in 10 and 20 μM ZD7288 (fig. 7d, e). Further quantitative analysis (fig. 7h) indicated that the average value of L$_{300}$ was 70.61 ± 0.82 ms for control C fibers, 68.56 ± 0.86% for 10 μM ZD7288, and 66.18 ± 0.88% for 20 μM ZD7288. The average MaxT$_{1}$ was 108.07 ± 0.67% for control C fibers, 109.87 ± 0.77% for 10 μM ZD7288, and 111.68 ± 0.79% for 20 μM ZD7288. In parallel, the average T$_{1, 300}$ was 106.24 ± 0.89% for control population, 107.94 ± 0.87% for 20 μM ZD7288 population, and 108.98 ± 1.01% for 20 μM ZD7288 population (p < 0.05, n = 8). Within the 22 fibers we examined, 12 C fibers exhibited the above-mentioned phenomenon, whereas 8 fibers exhibited no response, and 2 fibers a negative response (data not shown), suggesting that ZD7288 increases ISI change in a dose-dependent manner for type 3 C fibers.

**Discussion**

**New Types of C Fibers with Distinct Peripheral Processing**

A previous study by Weidner et al. [5] showed that in humans, a train of impulses reached the knee at a lower rate than was the frequency of the stimulus, when the neural activity level is low. In contrast, high neural background activity increased the intra-train frequency from 50 Hz to the entrained maximum of 160 Hz in a train of four pulses. They concluded that at a given degree of neural accommodation, all afferent C-units exhibit a uniform pattern of aftereffects that were independent of fiber class [5]. In the present experiments, three types of C fibers were observed in recordings from the saphenous nerve of rabbits. Only the basic change features observed

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**Fig. 6.** Effects of 4-AP on ISI change and activity-dependent conduction properties of type 3 C fibers in response to quintuplet pulse of 300 trains. a–c Representative ISI examples of a type 3 fiber induced by quintuplets of stimuli at an interstimulus interval of 20 ms were recorded before and 15 min after the application of 4-AP at different concentrations (control, 20, and 40 μM from top to bottom). The degree of ΔISI change for this type of C fiber decreased with the increase in the concentrations of 4-AP. Here, the ΔISI$_{1-2}$ exhibited the most significant reduction. d–f Changes in rCV of trailing AP$_1$ (T$_1$) and the last leading AP (L$_{300}$) induced by different concentrations (control, 20, and 40 μM from top to bottom). The activity-dependent slowing of type 3 C fibers decreased with increasing concentrations of 4-AP. g Summarized data for dose-response curves for 4-AP (n = 8). Both the MaxΔISI$_{1-2}$ and ΔISI$_{1-2}$EI reduced with the increase in different concentrations of 4-AP. h Summarized data for the effects of 4-AP on the changes in the rCV of trailing AP$_1$ and L$_{300}$. The rCV of MaxT$_{1}$ and T$_{1, 300}$ decreased with the increase in the concentrations of 4-AP, whereas the rCV of L$_{300}$ increased with the enhancement of concentrations of 4-AP (*, ** p < 0.05, n = 8). All data are represented as means ± SEM.

**AP Trains in Rabbit Saphenous Nerve Unmyelinated Fibers**

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ZD7288 (10 μM)

- CV ratio (%)
- ISI (ms)

Control

- CV ratio (%)
- ISI (ms)

ZD7288 (20 μM)

- CV ratio (%)
- ISI (ms)

Train (n)

- CV of the last leading AP
- CV of the first trailing AP

ZD7288 (10 μM)

- CV ratio (%)
- ISI (ms)

Train (n)

- CV of the last leading AP
- CV of the first trailing AP

ZD7288 (20 μM)

- CV ratio (%)
- ISI (ms)

Train (n)

- CV of the last leading AP
- CV of the first trailing AP
for type 3 C fibers (fig. 2c2, c3) resemble the results in Weidner et al.'s report. That is, in type 3 cells ISI1–2 was larger initially and then decreased to a stable interval compared to the interstimulus interval. In parallel, the ΔISI1–2 also changed from a short, positive period to a negative value in 'entrainment'. However, the characteristics of ISI1–2 for type 1 and type 2 C fibers observed in the present study were different from those of type 3, suggesting that all afferent C fibers do not exhibit a uniform pattern of aftereffects, which may depend on different intrinsic properties of C fibers.

Recently, Shim et al. [13] reported that two populations of C fibers showed different activity-dependent slowing of CV. One C fiber group exhibited a large amount of slowing of CVs and was presumed to consist of non-nociceptors, such as cold fibers, mechanoreceptors, and sympathetics. The other group exhibited a smaller degree of slowing of CVs and was presumed to consist of nociceptors. In the present study, these basic features of rCV may correspond to type 1 and type 3 C fibers (fig. 3a3, c3). However, the wavelike change in T1 from type 2 C fibers (fig. 3b3) has not been reported; this may represent a new type of C fiber.

**Possible Reason for the Marked Changes in ISI Restricted to the ISI1–2 of Train Series**

It is well known that the degree of CV slowing is closely related to the stimulus frequency and the interval between the condition impulses and test impulses [10, 14]. In addition, when the stimulus frequency is constant, the degree of change for aftereffects in the CV mainly depends on the interval between conditioning and test impulses. In the present study, C fibers were stimulated with trains of 5 pulses at a constant frequency (20 Hz) for 200 s. It was observed that the distinct changes in the ISI series were restricted to ISI1–2 and not present in the other three ISIs (ISI2–3, ISI3–4, and ISI4–5; fig. 2). We hypothesized that in an AP series with regular train patterns, the interval between conditioning and test impulses becomes the main factor for determining the degree of aftereffect on ISI. We believe that two kinds of aftereffects may be involved in regulating the ISI series. One is a 'longer-interval (>800 ms) aftereffect' from the preexisting trains, the other is a 'shorter-interval (about 50 ms) aftereffect' which occurred mainly within the intra-burst of the trains. In the train-firing series, the first AP of the burst (the leading AP) might mainly be subjected to the longer-interval aftereffect which induces a relatively smaller CV slowing. In contrast, the second AP of the burst (the trailing AP) might be influenced by the shorter-interval aftereffect as well as the longer aftereffect. This shorter-interval aftereffect may have a more prominent effect on activity-dependent slowing; thus, the combined action of these two kinds of aftereffects could result in a larger CV slowing. Taken together, ISI1–2 of the burst in the train series exhibits a greater change in interval, while the other three ISIs (ISI2–3, ISI3–4, and ISI4–5) experience less change because of their approximately equivalent aftereffects. Consistent with this result, Weidner et al. [5] reported that the response of a C fiber to quadruplets of stimuli at an interstimulus interval of 20 ms showed a larger change restricted to the ISI1–2. Furthermore, the above-mentioned phenomenon could be partially explained by the 'passive time constant' theory. An afterpotential is corresponding to (QNa – QK)/C, where QNa and QK is the quantity of Na⁺ and K⁺ ions flowing into the axon, and C is the capacitance of the membrane. When QNa = QK, all afterpotentials and post-spike velocity changes are absent due to the 'passive time constant' mechanism [9]. This may account for the lack of changes in the other three ISIs. Obviously, different stimuli protocols could alter the time order of aftereffect on the ISI series by changing the distribution of activity-dependent effects.

**Biophysical Properties Underlying Type-Specific ISI Change Patterns**

Specific patterns of activity-dependent changes in ISIs likely result from differences in the activity of many processes, including an activity-dependent time course of...
post-excitatory effects. We found that type 1 C fibers did not exhibit activity-dependent CV speeding as a sign of a supernormal period, which was apparent with the stimulus parameters used for the other two types of fibers. Because Weidner et al. [5] and Bostock et al. [9] found that some mechano-responsive (CMR) fibers have long-lasting time-dependent subnormality but not supernormality, we hypothesized that type 1 C fibers may be a kind of CMR fiber. In addition, we found that type 1 fibers showed less CV slowing than the other two types of C fibers. In the present study, type 2 C fibers exhibited both activity-dependent CV speeding and CV slowing, showing that ISI\(_{1-2}\) fluctuated around the stimulus interval. On the other hand, we found that type 2 fibers reached apparent CV slowing within 50 pulses after the onset, but the latency reversed partially during the remainder of the stimulation train (fig. 3b1, b3). Such a pattern of slowing is a characteristic of sympathetic efferents [18]. In addition, studies showed that recovery cycles of sympathetic units include both supernormal and subnormal velocities [9, 18]. Accordingly, we propose type 2 C fibers to be ‘sympathetic-like’, although additional study is needed. Furthermore, we found that ISI\(_{1-2}\) of type 3 C fibers increased for a shorter period initially, but then decreased at high levels of activity-dependent accommodation, which asymptotically approached the minimum ‘entrainment’ interval of the nerve fibers. This result is consistent with the pattern of ISI changes for CMR and heat-responsive (CMH) efferents in humans [5]. Accordingly, we propose that type 3 C fibers are CMH fibers. However, these suppositions may be open to different interpretations since other types of fibers, such as mechanically insensitive afferents and cold afferents, have not been examined in our studies.

**Ionic Mechanisms Underlying ISI Changes**

In the present study, we observed that application of 4-AP resulted in a decrease of ISI change (fig. 6). In some cases, at a higher concentration of 4-AP, all four ΔISIs were reduced approximately to zero. It is well known that Na\(^+\)-K\(^+\) pump activity can be markedly blocked by 4-AP in concentration- and voltage-dependent manners [19]. In addition, several studies have shown an inhibitory effect of 4-AP on voltage-gated tetrodotoxin-sensitive sodium currents [20]. Massive influx of sodium during repetitive spiking can activate the pump which in turn causes prolonged after-hyperpolarization. On the other hand, 4-AP can block K\(^+\) channels and reduce the K\(^+\) conductance [21]. In addition, 4-AP strongly facilitates the influx of calcium in unmyelinated axons [22]. The factors listed above can inhibit the pump activity directly or indirectly, reducing membrane hyperpolarization level, which then results in a decreased CV slowing. Therefore, the decrease of CV slowing may be the reason for the decrease in positive ΔISI at the initial times. Moreover, many studies have demonstrated that the presence of an activity-induced slowing of conduction is the prerequisite for CV speeding in the supernormal period, and its degree determines the amount of speeding [5, 9, 12]. Consequently, the decrease of CV slowing induced a subsequent decrease in negative ΔISI after the application of 4-AP.

In contrast, on the main axons of C fibers, blockade of \(I_{h}\) by its antagonist ZD7288 enhanced ISI change. A previous report indicated that ZD7288 enhanced the activity-dependent slowing of conduction seen in C fiber compound APs recorded from isolated rat vagus nerves and also augmented the post-tetanic hyperpolarization following trains of APs in unmyelinated and myelinated axons [23]. Consistent with this result, Soleng et al. [24] found that an activity-induced hyperpolarization could be partly countered by \(I_{h}\) in hippocampal Schaffer collaterals and concluded that \(I_{h}\) may play a role in maintaining excitability and reliable conduction during AP activity. In addition, hyperpolarization of the membrane produces different activation/inactivation states of other voltage-gated ion channels. For example, ZD7288 perfusion produces an after-hyperpolarization that accelerates the removal of inactivation from sodium channels, which allows more sodium ions to flow into the axon during repetitive spiking. The massive influx of sodium ions then activates the pump which in turn causes prolonged after-hyperpolarization. Taken together, these results suggest that the application of ZD7288 could result in further hyperpolarization of the axon, which leads to an increase of CV slowing and increase of ΔISI at early time. In support of this idea, we also found the subsequent increase of negative ΔISI and entrainment interval after ZD7288 perfusion.

**Functional Implications of Peripheral Signal Processing**

In previous studies, researchers paid close attention to the role of nociceptors and sensory neurons in pain generation [25, 26]. For example, some C fiber receptor terminals exhibit adaptation in response to a constant super-threshold stimulus. This kind of adaptation plays an important role in temporal summation mechanisms of tonic cutaneous mechanical pain [27]. Another example is neuropathic pain, which is characterized by spontaneous pain and tactile allodynia, i.e. pain evoked by normally innocu-
ous mechanical stimuli. Ectopic discharges developed in neurons of the dorsal root ganglion after peripheral nerve injury or inflammation are considered as painful signals for the generation and maintenance of neuropathic pain [17–29]. However, based on the results of the present study, the temporal pattern of APs may, in part, be due to the changes in ISIs during the conduction of spikes in C fibers. In other words, the temporal pattern of the response to natural stimuli may be modulated peripherally.

The fact that the axon modifies the coding of AP trains may explain the changes in the responses associated with various pathologies. For example, activation of axonal 5-HT (3) receptors, an important inflammatory mediator, not only enhanced membrane excitability, but also modulated AP trains in nociceptive C fibers at high impulse rates [6]. Uninjured C fibers in the L4 spinal nerve exhibited increased activity-dependent slowing following L5 spinal nerve injury in rats. The enhanced slowing can result in an increase in instantaneous frequency of a burst discharge when it reaches the spinal cord [30]. Altered properties of axonal excitability in amyotrophic lateral sclerosis induce a longer and irregular interval ectopic impulse, which causes fasciculations [31]. Regular high-frequency bursts occur when axons are strongly hyperpolarized by increased Na+ pumping. The regular high-frequency bursts are the reasons for the sensation of ‘pins and needles’ following a prolonged period of limb ischemia [32]. Demyelinating diseases, such as multiple sclerosis, are associated with both negative and positive symptoms. Positive symptoms are caused by regular ectopic discharges from demyelinated axons perceived as paresthesia [33]. Based on these data, we hypothesize that in some pathological conditions the adaptation properties may be perturbed by altering both numbers and patterns of firing series, ultimately influencing perception or movement. In the present study, we found the peripheral process of firing series can change temporal structure in different types of C fibers, which strongly suggests that the function of information processing could also occur in the propagation course along the main axon of C fibers.

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