Activity-Dependent Modulation of Axonal Excitability in Unmyelinated Peripheral Rat Nerve Fibers by the 5-HT(3) Serotonin Receptor

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INTRODUCTION

Action potentials in human nociceptive nerve fibers have been studied in detail by microneurography (Handwerker 1996). One key conclusion made by using this technique is that the conscious sensation of pain arises as the result of a series rather than of a single nerve action potential. For example, CO2 laser stimuli were perceived as painful only when the spike trains in nociceptive units lasted for >60 ms (Bromm et al. 1984). In this study of Bromm and colleagues, interspike intervals in the trains of impulses had a minimum at 1984). In this study of Bromm and colleagues, interspike intervals in the trains of impulses had a minimum at 1 Hz or in hyperpolarized axons, mCPBG produced a loss of superexcitability. In contrast, at 0.33 Hz, a small increase in the postspike subexcitability was observed. Similar effects on excitability changes were found when latency instead of threshold was recorded, but only at higher action potential frequencies: at 1.8 Hz, mCPBG increased conduction velocity and reduced postspike supernormality. The latter effect would increase the interspike interval if pairs of action potentials were conducted along several cm in an inflamed nerve trunk. These data indicate that activation of axonal 5-HT(3) receptors not only enhances membrane excitability but also modulates action potential trains in unmyelinated, including nociceptive, nerve fibers at high impulse rates.

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2002). Furthermore, mice deficient for the serotonin transporter did not develop thermal hyperalgesia after chronic constrictive sciatic nerve injury (Vogel et al. 2003).

The excitability of unmyelinated axons in isolated short segments of peripheral mammalian nerve can be measured with high sensitivity by means of a computer-controlled threshold tracking method originally written for studies of human nerves in vivo (Bostock et al. 1998; Burke et al. 2001). Previously, this method was used to follow changes in membrane excitability during application of a variety of neuropeptides including mediators involved in inflammatory and neuropathic pain (Irnich et al. 2002; Lang et al. 2003; Moalem et al. 2005). In the present study, we have applied the threshold tracking method to study the changes in excitability that occur in the recovery period after single action potentials in peripheral C-fibers. These recordings of the postspike excitability cycle are supplemented by measurements of latency shifts in the recovery period. We demonstrate that activation of the ionotropic 5-HT(3) receptor by m-chlorophenylbiguanide (mCPBG) alters the postspike recovery cycle and causes activity- and potential-dependent neuromodulation of axonal excitability. We also describe that mCPBG has frequency-dependency effects on changes in C-fiber conduction velocity. One of the consequences of these alterations is that interspike intervals will be prolonged when pairs of action potentials are conducted along several cm of unmyelinated axons in a nerve trunk with raised extracellular concentration of serotonin. This might be functionally significant in neuropathies or nerve injuries accompanied by inflammation along the nerve trunk.

METHODS

Animals and preparation

Adult Wistar rats, weighing 250–350 g (University of Munich or Biological Resources Center, University of New South Wales, Sydney, Australia), were used in the experiments. All procedures were approved by federal law (Germany) or by the Animal Care and Ethics Committee of the University of New South Wales. Sural nerves were removed from Wistar rats that were either deeply anesthetized and subsequently killed with an overdose of sodium pentobarbital (120 mg/kg ip; Sydney) or from rats after death due to asphyxiation in a chamber filled with carbon dioxide gas (Munich). The isolated nerves were immediately put into fresh artificial cerebrospinal fluid (ACSF) solution containing (in mM) 117 NaCl, 3.6 KCl, 2.5 CaCl₂, 1.2 MgCl₂, 1.2 NaH₂PO₄, 25 NaHCO₃, and 11 D-glucose; pH 7.4 (bubbled with 95% O₂-5% CO₂). The nerves were desheathed under a microscope and then held at each end by suction electrodes in an organ bath. One suction electrode was used to elicit action potentials, while the other was used as a recording electrode. The distance between stimulating and recording electrodes was ~3.7 mm. The organ bath (volume: about 1 ml) was continuously perfused with ACSF solution at a flow rate of 8 ml/min and a temperature of 30°C. All chemicals, including mCPBG, were purchased from Sigma (Taufkirchen, Germany).

Electrophysiological recordings

Axonal excitability parameters and latency recordings were performed using the QTRAC program (Institute of Neurology, London, UK). QTRAC is a flexible, stimulus-response data-acquisition program, originally written for studies of human nerves in vivo (Bostock et al. 1998) but also suitable for electrophysiological recordings from isolated peripheral nerves. In the present study, QTRAC was used to record compound action potentials (CAPs) from peripheral C-fibers, to generate stimuli, and to display the results. The isolated segments of sural nerves were stimulated with a linear stimulus isolator (A395, WPI, Sarasota, FL) with a maximal output of 1 mA. The stimulator was controlled by a computer via a data-acquisition board. Nerve excitability and latency were tested with 1-ms current pulses at different frequencies. For threshold tracking experiments, the stimulus strength was automatically adjusted to maintain the C-fiber CAP at a constant amplitude (40% of the maximum, defined as “threshold”). In latency recordings, CAPs of maximal amplitude were used. Because axons being tracked at threshold are excited on average by 50% of the test stimuli, the effective stimulation rate for threshold-tracking experiments was calculated as the number of supramaximal stimuli plus half the number of test stimuli per repeat stimulation sequence, divided by the length of the sequence in seconds. In a typical stimulation sequence, successive sweeps delivered every 1 or 3 s contained: a supramaximal stimulus (to monitor peak response amplitude), a test stimulus alone, and a supramaximal conditioning stimulus + test stimulus. This sequence resulted in a mean stimulation rate for axons at threshold of 1 + 1/2 +1/2 stimuli every 3 or 9 s (1 or 0.33 Hz). In the case of a five-channel protocol (see Fig. 3), 1 + 1/2 + 1/2 + 1/2 + 1/2 stimuli were given every 5 or 15 s (1 or 0.33 Hz).

Statistical analysis

Data were analyzed using paired or unpaired Student’s t-test as appropriate. The levels of significance are given by: * P < 0.05, ** P < 0.01, *** P < 0.001.

RESULTS

Postspike excitability of C-fibers depends on stimulation rate

In initial experiments, the importance of the stimulation rate for changes in axonal excitability following a conditioning action potential, i.e., the recovery cycle of excitability in unmyelinated axons, was tested. Isolated segments of rat sural nerves were tested using three stimulus conditions (channels) of the program QTRAC: (channel 1) a constant supramaximal stimulus evoked the maximum peak-to-peak amplitude of the C fiber component of the CAP; (channel 2) a target response was set at 40% of the maximum response on channel 1, and the test stimulus current (“threshold current”) was adjusted by computer to maintain the response close to the target; (channel 3) same protocol as for channel 2, but a conditioning supramaximal stimulus preceded the test stimulus (Fig. 1A). These three stimulus conditions were repeated continuously with either an interval of 3 or 1 s between the successive stimulation sweeps (effective stimulation frequency: 0.33 or 1 Hz; see METHODS). Changes in axonal excitability following a C-fiber CAP of maximal amplitude (i.e., the recovery cycle of excitability) were determined by comparison of the current necessary for a CAP with 40% of the maximum amplitude without (test stimulus only, channel 2) and with a conditioning stimulus of maximal amplitude (conditioning plus test stimulus, channel 3). The difference in these currents were determined for interspike intervals between the conditioning and the test stimuli of 10–100 ms.

A representative example of this type of experiment and the statistical analysis is illustrated in Fig. 1. At a stimulation frequency of 0.33 Hz, a long-lasting period of subexcitability was measured in the postspike recovery period. In contrast, axonal superexcitability was observed when the sural nerve...
preparations were stimulated with a stimulation frequency of 1 Hz. Changes of excitability in the postspike period were determined at the two stimulation frequencies in five different sural nerve preparations. Differences between 0.33 and 1 Hz are statistically significant for all tested intervals ($P < 0.01$, $P < 0.05$ at 10-ms intervals; Fig. 1C).

The importance of stimulation rate for changes in excitability during the postspike period was also tested by continuous threshold tracking at a fixed interval of 25 ms between the conditioning and the test CAP. A representative example for such an experiment is illustrated in Fig. 1B. In this recording, the baseline stimulation frequency of 0.33 Hz was increased to 1 Hz for 3 min. The increase in membrane threshold observed during the period of higher action potential activity was accompanied by a reversal from axonal sub- to superexcitability in the postspike period. Quantitatively, an inversion of membrane subexcitability from $14.4 \pm 2.3\%$ ($n = 5$; mean $\pm$ SD) at 0.33 Hz to superexcitability of $-12.4 \pm 4.0\%$ ($n = 5$) at 1 Hz was observed.

Action potential frequency alters the effects of mCPBG

The effects of mCPBG, an agonist at axonal 5-HT(3) receptors, on axonal excitability and the postspike recovery period were tested at two different stimulation rates on C-fibers in rat sural nerve segments. A representative example for these experiments is illustrated in Fig. 2. The recording was started with a stimulation frequency of 1 Hz. At this stimulation rate, axonal superexcitability of $\sim 10\%$ was measured 30 ms after the conditioning compound C-fiber action potential. Addition of mCPBG into the bathing solution reduced membrane threshold by $\sim 16\%$ (reduction in threshold current from 385 to 325 $\mu$A) and produced a loss of postspike superexcitability. Afterward, the stimulation frequency was reduced to 0.33 Hz. This resulted in a reduction of membrane threshold and the development of postspike subexcitability (see also Fig. 1). Application of mCPBG during this condition resulted in an increase in excitability of only 5% (reduction in threshold current from 315 to 300 $\mu$A). In addition, the postspike subexcitability was slightly enhanced. This type of experiment was performed on several sural nerve fascicles (also with the opposite order of stimulation frequencies). The statistical analysis is illustrated in Fig. 4. The data reveal significant differences in the effects of mCPBG at low compared with high stimulation rates. In axons with high action potential frequency, mCPBG produced a large increase in axonal excitability accompanied by a reduction or loss of postspike superexcitability. In contrast, only...
a small reduction of threshold and an increase of the subexcitability in the recovery period was observed when the effects of mCPBG were tested at the low stimulation rate.

Importance of membrane potential.

The importance of action potential frequency on the postspike recovery period and on the increase in axonal excitability produced by mCPBG may be either due to the number of action potentials or due to activity-dependent changes in membrane potential. Therefore we recorded the postspike excitability during a constant stimulation frequency but at two different axonal membrane potentials. For this type of experiment, the threshold tracking protocol was extended by two additional channels. A typical observation made by using this five-channel threshold tracking protocol on an isolated rat sural nerve segment is illustrated in Fig. 3. Postspike excitability at the “resting” membrane potential was determined using channel 2 (current necessary for a target response set at 40% of the maximum peak amplitude of the CAP) and channel 3 (same parameter 30 ms after a conditioning maximal CAP). In addition, in channels 4 and 5, postspike excitability was determined during membrane hyperpolarization induced by application of current pulses with an onset 50 ms before the test pulse (amplitude: −10% of current used in channel 1; duration: 100 ms).

By using this threshold tracking protocol, it is revealed that postspike excitability depends on membrane potential and not on stimulation frequency. The example in Fig. 3 illustrates that the rat C-fiber axons stimulated at a frequency of 0.33 Hz are subexcitable at a stimulus interval of 30 ms (see threshold tracking channels 2 and 3). However, postspike superexcitability was observed during application of hyperpolarizing pulses (see threshold tracking channels 4 and 5). Quantitatively, postspike excitability at the “resting” potential and during membrane hyperpolarization were determined in four rat sural nerve segments at a stimulation frequency of 0.33 Hz (interspike interval: 30 ms). Postspike subexcitability of 10.8 ± 3.1% (n = 4) was found at the resting potential. In contrast, membrane hyperpolarization produced axonal postspike superexcitability of −8.3 ± 2.5% (n = 4).

The five-channel threshold tracking protocol was also used during bath application of mCPBG (Fig. 3). We observed that membrane hyperpolarization, similar to the situation of a high action potential frequency, enhanced the mCPBG-induced increase in axonal excitability. Also, there was a strong reduction of postspike superexcitability when mCPBG was applied at a low stimulation rate but during a hyperpolarizing prepulse. A summary of these observations and the statistical analysis is illustrated in Fig. 4.

Effects of mCPBG on the postspike latency recovery period

Changes in latency during the postspike recovery period were determined using CAPs of maximal amplitude. A representative example for such experiments is illustrated in Fig. 5. The shape of the CAP differed strongly at the various stimulation rates. A rather uniform configuration was observed at 0.6 Hz. However, a segregation in an early and a late component was produced by an increase in the action potential frequency from 0.6 to 1.8 Hz (Fig. 5A). Addition of conditioning action potentials revealed frequency-dependent shifts in latency (Fig. 5B). Axonal conduction velocity was reduced (subnormal) 40 ms after a prepulse during a mean stimulation rate of 0.6 Hz. In contrast, a shorter latency (supernormal) to the late component in the CAP was found when the conditioning action potential was tested at 1.8 Hz.

The effects of mCPBG on the postspike latency recovery period were tested using a five-channel stimulus protocol. In all five channels of this protocol, a test stimulus with a compound C-fiber action potential of maximal peak height
was generated. In four of the stimulus channels, a single conditioning action potential of maximal amplitude was added with interspike intervals of 100, 40, 20, and 12 ms between the conditioning and the test stimulus. The effects of mCPBG (3 μM) were studied at different stimulation frequencies by using the latency to 50% of the test CAP peak amplitude as the recording parameter. A representative example for an observation made using a stimulus rate of 1.8 Hz and an interspike interval of 40 ms is illustrated in Fig. 6. A clear reduction in latency is revealed by superposition of the unconditioned and the conditioned test action potential (Fig. 6A). This difference in latency was strongly reduced in the presence of mCPBG (Fig. 6B). Details and the time course of this effect were observed by continuous recording of latencies as shown in Fig. 6B. The recording illustrates that the supernormality in the recovery period would produce a reduction of the interspike interval from 50 to ~20 ms after the conduction distance of 20 cm. However, in the presence of mCPBG, the interspike interval would be still 40 ms as compared with only 20 ms seen in the control condition.

**DISCUSSION**

Activity-dependent changes in latency, threshold, and postspike recovery cycle are well documented for rat (Gee et al. 2003) and in human (Bostock et al. 2003) C-fibers. The changes in the postspike latency recovery period produced by mCPBG during a stimulation frequency of 1.8 Hz are superimposed. Note the increase in latency due to reasons discussed later. The calculated data plotted in Fig. 7B indicate that the supernormality in the recovery period would produce a reduction of the interspike interval from 50 to ~20 ms after the conduction distance of 20 cm. However, in the presence of mCPBG, the interspike interval would be still 40 ms as compared with only 20 ms seen in the control condition.

**FIG. 4.** Overview of activity- and potential-dependent effects of mCPBG such as illustrated in Figs. 2 and 3. A: changes in membrane threshold observed during application of mCPBG (3 μM) at low (0.33 Hz) and high (1 Hz) stimulation rate and during a combination of low stimulation rate and a hyperpolarizing prepulse are summarized (means ± SD). Note that the strongest reduction in threshold (increase in excitability) was seen when mCPBG was added during the high stimulus rate. Membrane hyperpolarization, too, enhances the effect of mCPBG. B: summary of postspike excitability (interspike interval: 30 ms) measured before and during application of mCPBG (3 μM) in the 3 experimental conditions (means ± SD). Note that mCPBG reduces postspike excitation at the lower stimulus rate and slightly increases. Membrane hyperpolarisation during stimulation with 0.33 Hz produces effects of mCPBG similar to a stimulus rate of 1 Hz. (number of experiments are given in each column; **P < 0.01; ***P < 0.001).

**FIG. 5.** Activity-dependent changes in the shape of the compound C-fiber action potential. A: repetitive stimulation of a sural nerve branch with 0.6 or 1.8 Hz. The higher stimulation rate produces a segregation of the compound action potential into 2 discrete (early and late) components. B: effects of a conditioning prepulse on latency. A compound C-fiber action potential of supra-maximal amplitude was elicited 40 ms before the test pulse (see inset for the stimulus protocol). The unconditioned and the conditioned test pulse during stimulation with 0.6 or 1.8 Hz are superimposed. Note the increase in latency (subnormality) in the postspike recovery period at 0.6 Hz. In contrast, a clear reduction in latency (supernormality) of the late component is produced by the conditioning prepulse at 1.8 Hz.
al. 1996; Shin and Raymond 1991; Thalhammer et al. 1994) and human (Bostock et al. 2003; Serra et al. 1999; Weidner et al. 2002) C-fiber axons. In most of these studies, single-fiber recordings in situ provided a method for correlation of electrophysiological parameters with the function of the C-fiber. Our experimental observations are based on recordings of CAPs from isolated short rat sural nerve segments in an organ bath. This type of recording allows stable electrophysiological recordings (for up to several hours) and application of inflammatory mediators to several millimeters of desheathed nerve segments. We confirm several observations previously made with single-fiber recordings in peripheral rat nerves such as changes of both threshold and conduction velocity following impulse activity (Shin and Raymond 1991) and differences in activity-dependent slowing of different classes of C-fibers (Gee et al. 1996; Thalhammer et al. 1994). The new finding is that an agonist at the 5-HT(3) receptor changes these activity-dependent electrophysiological parameters (including the superexcitability and supernormality in the postspike recovery period) of C-fiber axons.

Several previous studies revealed that trains of action potentials in axons are modified by voltage-dependent ion channels, changes in intra- or extracellular ion concentrations, and variations in structural parameters such as the axonal diameter (Debanne 2004). However, there is much less literature about the importance of axonal chemosensitivity for the conduction of bursts of action potentials and the functional consequences of this type of neuromodulation. Axonal information processing has been found in studies using recordings from crustacean axons. For example, focal application of dopamine to motor axons evokes additional action potentials in intervals of spike bursts generated in the soma of the motor neuron. Thus the peripheral axon can shape the output signaling to the muscles (Bucher et al. 2003). Also axons of neurons in command and integrating centers of the crustacean nervous system are activated by neuroligands such as octopamine (Goaillard et al. 2004). This effect can modulate the central pattern generating network of the pyloric rhythm. An example for axonal information processing in mammalian sensory axons has been found along central axons of trigeminal muscle spindle affer-
ents involved in jaw movements (Verdier et al. 2003). Propagation of antidromic spikes in these sensory neurons is controlled by GABA_A receptors located on the caudal axon. Data in the present study demonstrate that modulation of axonal excitability by serotonin can prolong the interspike interval in unmyelinated axons of a mammalian peripheral nerve trunk. This effect might alter the perception of a stimulus applied to the peripheral terminal of a nociceptive neuron.

Possible function of fibers with effects of mCPBG

Different classes of C-fibers contribute to the shape of the CAPs recorded in the present study. The function of the fibers affected by mCPBG are not clearly revealed under these circumstances. However, a segregation of the compound potential into two components was observed at higher action potential frequencies (see Fig. 5). In single-fiber recordings described by other authors, differences in activity-dependent slowing of the various functional classes of C-fibers in peripheral rat and human nerve have been observed. According to these data, there is more activity-dependent reduction of conduction velocity in unmyelinated nociceptive as compared with nonnociceptive (e.g., cold) fibers (Gee et al. 1996; Serra et al. 1999; Thalhammer et al. 1994). In human skin nerve, single sympathetic C-fiber efferents have also been studied. These fibers, too, showed much less activity-dependent slowing as compared with nociceptive axons (Campero et al. 2004). Previously, we demonstrated that sympathetically does not influence the effect of mCPBG on axonal excitability in sural nerve of the rat, indicating an effect on sensory C-fiber afferents rather than postganglionic sympathetic efferents (Moalem et al. 2005). In the present study, mCPBG produced a reduction in latency (increase in conduction velocity) of the late component in the CAP recorded at high stimulation rates (see Fig. 5). This indicates that mCPBG modulates the excitability of nociceptive C-fiber axons.

The use of CAPs in threshold tracking experiments has the disadvantage that changes in peak height may occur due to alterations in the conduction velocities of the constituent nerve fibers. For this reason, the maximal response of the compound potential was measured before each test stimulus. This procedure ensures that the determination of the threshold current is based on a signal with 40% of the actual maximal amplitude and not of a fixed value set at the beginning of the recording period. Any changes in peak height due to alterations in conduction velocity are thereby compensated appropriately, provided conduction slowing is similar for the fibers contributing to the maximal peak and those contributing to the 40% level.

Modulation of superexcitability and supernormality

Both the reductions in threshold (superexcitability) and in latency (supernormality) observed in the postspike recovery period at action potential frequencies ≥1 Hz are consequences of a long-lasting depolarizing afterpotential. At present, based on detailed microneurographic studies of single human axons, the depolarizing afterpotential of C-fibers is explained as a passive electrical phenomenon (Bostock et al. 2003; Weidner et al. 2002). Previously, this hypothesis has been used to model the depolarizing afterpotential in amphibian A-fibers (Barrett and Barrett 1982). According to this model, an action potential produces a long-lasting passive depolarizing afterpotential if the axonal membrane has a long membrane time constant. In fact, Bostock and colleagues (2003) determined a mean value of 110 ms for the membrane time constant of human C-fiber axons. The postspike afterpotential is given roughly by \( (Q_{Na} - Q_K)/C \), which decays with a time constant given by membrane resistance and membrane capacitance. \( Q_{Na} \) and \( Q_K \) describe the number of Na⁺ and K⁺ ions that pass through voltage-dependent sodium and potassium channels during the action potential; \( C \) is the membrane capacitance. Whether the afterpotential is de- or hyperpolarizing is determined by the voltage dependence of \( Q_{Na} \) and \( Q_K \), rather than by the membrane resistance, which in human C-nociceptive fibers is fairly constant at stimulation frequencies of ≥0.25 Hz (Bostock et al. 2003). This model can explain why an increase in the stimulation rate produces a change from postspike sub- to superexcitability (see Fig. 1) and from postspike sub- to supernormality (see Fig. 7), respectively. The most likely reason is that hyperpolarization due to electrogenic sodium pumping enhances the inward sodium current (i.e., more \( Q_{Na} \)) and reduces the outward potassium current (less \( Q_K \)) during an action potential. The stimulation frequency itself, on the other hand, does not seem to cause this phenomenon. Our observations made by using a five-channel threshold tracking protocol (Fig. 3) support this view. Both, sub- and superexcitability can be recorded at stimulation frequencies <1 Hz when the recovery period is tested at the “resting” and a hyperpolarized membrane potential.

Application of mCPBG [agonist at 5-HT(3) receptors] strongly reduced postspike superexcitability and supernormality. The presence of 5-HT(3) receptors on peripheral nociceptive nerve fibers is well documented (Beck and Handwerker 1974; Zeitz et al. 2002). In particular, the depolarizing effect of 5-hydroxytryptamine and mCPBG on unmyelinated axons in vagus nerve preparations has been studied in much detail (Elliott and Wallis 1990; Kilpatrick et al. 1990). A cationic inward current through the ionotropic 5-HT(3) subtype of serotonin receptor (Hoyer et al. 2002) produces this effect. Membrane depolarization seems to be also the main mechanism underlying the mCPBG-induced increase in axonal excitability of unmyelinated nerve fibers in rat sural nerve (see Fig. 2). An example of a functional consequence is the finding that serotonin sensitizes acutely axotomized nerve fibers to mechanical stimuli (Michaelis et al. 1998). Effects of serotonin have been also observed on motor axons. In this case, modulation of crustacean motor axons by serotonin is likely to influence the strength and duration of muscle contraction (Meyrand et al. 1992). A further aspect in the modulation of axonal function by serotonin described in the present study is the reduction of superexcitability and supernormality in the recovery period. These effects can be explained by the model described in the preceding text. Membrane depolarization produces a voltage-dependent decrease in the inward sodium current (less \( Q_{Na} \)) and an increase in outward potassium current (more \( Q_K \)) during the action potential. As a consequence, the depolarizing afterpotential, and the postspike superexcitability and supernormality are reduced.
Effects of mCPBG on C-fiber axons at low action potential frequency

During a stimulation rate of <1 Hz, mCPBG only induced a small increase of axonal excitability (see Fig. 4). This difference to the effect observed at higher action potential frequencies (≥1 Hz) can be explained by the relationship between membrane potential and threshold current of peripheral axons (e.g., Fig. 11 in Bostock and Grafe 1985). This U-shaped relationship is based on the voltage dependency of ion channels that contribute to the threshold of the axonal membrane. As a consequence, membrane depolarization has less effect on threshold at a relatively low as compared with a high “resting” potential. We also observed that mCPBG slightly enhanced the subexcitability observed during the recovery period at low action potential frequencies (<1 Hz). According to the model described in the preceding paragraph, postspike subexcitability is generated by a long-lasting hyperpolarizing afterpotential due to a capacitative net outward current produced when $Q_{\text{Na}}$ exceeds $Q_{\text{K}}$ during the action potential at a low membrane potential. In the presence of mCPBG, membrane depolarization results in a reduction of $Q_{\text{Na}}$ due to voltage-dependent inactivation of Na$^+$ channels. In addition, a voltage-dependent increase in outward potassium current (more $Q_{\text{K}}$) is produced. The consequence of these effects is an increase in postspike subexcitability.

Relevance of findings for pathophysiology of nerve trunk inflammation

We observed that activation of 5-HT(3) receptors alters axonal excitability and conduction velocity in the postspike recovery period of unmyelinated axons in a peripheral rat nerve. This effect is more marked in active axons, i.e., at action potential frequencies >1 Hz. It is likely that such changes in electrophysiological parameters have consequences for the axonal function in a condition such as nerve trunk inflammation. The fact that mCPBG is less effective at the axonal “resting” potential (low rate of action potentials) indicates that the activation of 5-HT(3) receptors results in modulation of action potential series rather than in recruitment of resting fibers. The overall effect of the various changes in excitability is an increase in threshold current necessary for initiation of a second action potential. In latency recordings, this effect produces a clear increase in the interspike interval if pairs of action potentials are conducted along long nerve segments (see Fig. 7). Interestingly, this phenomenon has been seen in human C-fibers after skin inflammation (Olausson 1999b).

The consequences of postspike supernormality for the conduction of impulse series have been documented using single fiber recordings from peripheral human C-fibers. In a study by Weidner and colleagues (2002), the output frequency (interspike interval) recorded at knee level was compared with the input frequency (interstimulus intervals of 20 or 50 ms) applied to the receptive field at the foot. At higher action potential frequencies, interspike intervals decreased because of the supernormal period to a minimum of ~11 ms. Our data indicate that inflammatory mediators released during nerve trunk inflammation may alter this activity-dependent modulation of action potential series. The calculation plotted in Fig. 7B results in the conclusion that activation of the 5-HT(3) receptor by mCPBG produces much less activity-dependent reduction of the interspike interval. However, we would like to emphasize that the accuracy of this calculation is limited due to the use of isolated short nerve segments. First, it is difficult to measure a conduction velocity because the exact site of action potential generation in the tip of the suction electrode is unknown. Second, we have used a bath temperature of ~30°C, which is below the in vivo condition. Nevertheless, the data indicate clear changes in the interspike interval when trains of impulses are conducted at high action potential frequencies along a long stretch of a depolarized C-fiber axon. We demonstrate that this effect may be produced by serotonin on axons with pronounced activity-dependent slowing, i.e., nociceptive nerve fibers.

Changes in interspike intervals and/or the duration of a train of action potentials may modify temporal summation and/or long-term potentiation at the synaptic contacts of nociceptive fibers in the dorsal spinal horn (Sandkühler and Liu 1998). This effect might modify the perception of a stimulus applied to the peripheral terminals of a primary afferent nociceptive neuron. Also it has been suggested that the activity-dependent reversal from sub- to superexcitability in the recovery period may enhance the contrast between nociceptive stimuli of low and high intensity (Weidner et al. 2002). According to this view, the loss of superexcitability and supernormality produced by activation of the 5-HT(3) receptor may lead to a decrease in the ability to discriminate different stimulus strengths.

In summary, our data indicate the possibility that mediators in the inflammatory milieu such as 5-HT(3) contribute to symptoms in nerve trunk inflammation, not only by lowering the threshold for spike initiation but also by altering the interspike intervals in trains of action potentials.

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