Prostaglandin E2 Has No Effect on Two Components of Tetrodotoxin-Resistant Na+ Current in Mouse Dorsal Root Ganglion

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Abstract. One possible mechanism underlying inflammation-induced sensitization of the primary afferent neuron is the upregulation of tetrodotoxin-resistant (TTX-R) Na+ current by inflammatory mediators such as prostaglandins. This notion is based on reports that showed an augmentation of TTX-R Na+ current following an application of prostaglandin E2 (PGE2) in dorsal root ganglion (DRG) neurons. However, no information was available on the properties of the novel type of TTX-R Na+ channel, NaV1.9, at times when these reports were published. Hence, the contribution of NaV1.9 to the PGE2-induced upregulation of TTX-R Na+ current remains to be elucidated. To further examine the modulation of TTX-R Na+ current by PGE2, we recorded two components of TTX-R Na+ current in isolation from small (<25 µm in diameter) DRG neurons using wild-type and NaV1.8 knock-out mice. Unexpectedly, neither the component mediated by NaV1.8 nor the persistent component mediated by NaV1.9 was affected by PGE2 (1 and 10 µM). Our results raise a question regarding the well-known modulatory role of PGE2 on TTX-R Na+ current in inflammatory hyperalgesia.

Keywords: prostaglandin E2, tetrodotoxin-resistant Na+ current, dorsal root ganglion, inflammatory hyperalgesia, patch clamp recording

Introduction

Voltage-gated Na+ channels mediate a rapid and transient increase in Na+ permeability in response to changes in membrane potential, thereby contributing to the generation and conduction of action potentials that serve as sensory signals from the periphery to the spinal cord through the primary afferent neurons. The primary afferent neurons with their cell bodies in the dorsal root ganglion (DRG) express two classes of Na+ currents that can easily be separated pharmacologically on the basis of sensitivity to tetrodotoxin (TTX), one blocked by nanomolar TTX and the other resistant to micromolar TTX.

The TTX-resistant (TTX-R) Na+ current that is observed preferentially in smaller types of DRG neurons (1 – 3) can be subdivided into the component mediated by NaV1.8 (known as SNS/PN3) (4 – 7) and the persistent component mediated by NaV1.9 (known as NaN/SNS2) (8 – 12). The former component (which we refer to as ISNS) activates and inactivates more slowly than TTX-sensitive Na+ currents (4, 5, 13) and is thought to play a key role in action potential electrogenesis in small DRG neurons (7, 13). The latter component (referred to as INaN) is characterized by extremely prolonged time courses of activation and inactivation at low activation voltages (9, 12, 14) and may regulate subthreshold excitability of small DRG neurons (9, 11 – 13).

Tissue damage results in inflammatory hyperalgesia, associated with an increase in excitability of the primary afferent neurons. It has originally been shown that the amplitude of TTX-R Na+ current in the primary afferent neurons is upregulated by prostaglandin E2 (PGE2), an inflammatory mediator (15, 16). These observations led to the hypothesis that altered function of the TTX-R Na+ channel is responsible for the hyperexcitability of the primary afferent neurons that may contribute to inflammatory hyperalgesia (17 – 24). Thus, PGE2-induced...
upregulation of TTX-R Na⁺ current is now widely accepted as one of the important mechanistic bases for the prostaglandin-induced hyperalgesia (25).

However, the precise mechanism by which PGE₂ enhances TTX-R Na⁺ current has not been fully investigated. In addition, TTX-R Na⁺ current was generally thought to be a homogeneous current and little or no information was available on the properties of I_SNS at times when a majority of the papers that reported the role of TTX-R Na⁺ current in inflammatory pain were published. Even after physiological characterization of I_SNS (12–14), the target Na⁺ current has been collectively treated as “TTX-R Na⁺ current” without further segregation of the current (23). Consequently, a possible involvement of I_SNS has not been taken into consideration. In this study, therefore, we investigated the effect of PGE₂ on isolated I_SNS or I_SNN using wild-type (WT) as well as the Na⁺-1.8 knock-out (KO) mice (6, 12, 13). Unexpectedly, PGE₂ had no detectable effect on I_SNS or I_SNN, raising a question regarding the modulatory role of PGE₂ on TTX-R Na⁺ current in inflammatory hyperalgesia.

Materials and Methods

Isolation of single DRG neurons and cell culture

All procedures were carried out according to protocols approved by Hiroshima University Animal Ethics Committee. The dissociation of single DRG neurons and their culture have been described previously (12). Briefly, adult mice were sacrificed by cervical dislocation during ethylcarbamate (3 mg/kg; Wako Pure Chemicals, Osaka) anesthesia. The DRGs from all spinal levels were removed and desheathed in ice-cold, Ca²⁺/Mg²⁺-free, phosphate-buffered saline. The isolated DRGs were enzymatically digested at first with 0.2% collagenase (Wako Pure Chemicals) and then with 0.1% trypsin (Sigma, St. Louis, MO, USA), each for 20 min at 37°C. The DRGs were then dissociated by titration with fire-polished Pasteur pipettes, and cells were plated onto glass coverslips coated with 0.01% poly-L-lysine (Sigma).

The dispersed DRG neurons were maintained in Dulbecco’s modified Eagle medium (Gibco, Grand Island, NY, USA) supplemented with 10% (vol/vol) heat inactivated fetal bovine serum (Gibco), penicillin (100 IU/ml, Sigma), and streptomycin (100 µg/ml, Sigma) at 36°C in a humidified incubator, which contained 5% CO₂ in air. Cells under short-term culture (4 to 12 h after plating) were used for experiments. At this time in culture, neurite outgrowth was not observed. We defined DRG neurons that were smaller than 25 µm in diameter as small neurons (26), and small neurons thus defined were used throughout the study. We employed KO DRG neurons (6, 12, 13) in addition to WT DRG neurons in this study. Unless otherwise specified, the experiments were performed in neurons from WT DRG.

Electrophysiology

We performed voltage-clamp recordings using an Axopatch 200A amplifier (Axon Instruments, Union City, CA, USA). Data were low-pass-filtered at 5 kHz with a four-pole Bessel filter and digitally sampled at 25–100 kHz. In some experiments, capacitive and leakage currents were subtracted digitally by the P–P/4 procedure (5). We recorded membrane currents using either the ‘conventional’ whole-cell patch-clamp technique (27) or the nystatin-perforated patch-clamp technique (28). The patch pipette solution contained 10 mM NaCl, 110 mM CsCl, 20 mM tetraethylammonium (TEA)-Cl, 2.5 mM MgCl₂, 5 mM HEPES, 5 mM EGTA, and 3 mM Mg²⁺-ATP. Since a part of the recordings were performed with a pipette solution that did not contain Mg²⁺-ATP, only traces recorded without Mg²⁺-ATP were specified. The pH of the pipette solution was adjusted to 7.0 with CsOH. Osmolarity was adjusted to 290 mosmol/kg with glucose. For the patch pipette solution of nystatin-perforated patch recordings, a stock solution containing 10 mg/ml nystatin (Wako Pure Chemicals) was prepared and added to the pipette solution to reach a final concentration of 500 µg/ml. The DC resistance of patch electrodes was 0.8–1.5 MΩ for the conventional whole-cell patch and 2–3 MΩ for the nystatin-perforated patch, respectively.

The external solution contained 100 mM NaCl, 5 mM CsCl, 30 mM TEA-Cl, 1.8 mM CaCl₂, 1 mM MgCl₂, 0.1 mM CdCl₂, 5 mM HEPES, 25 mM glucose, and 5 mM 4-aminopyridine (4-AP). TEA-Cl and 4-AP were added to abolish K⁺ currents and Cd²⁺ was added to abolish Ca²⁺ currents (5). The pH of the external solution was adjusted to 7.4 with HCl (since 4-AP is strongly alkaline). Osmolarity was adjusted to 290 mosmol/kg with glucose. We compensated for the liquid junction potential between internal and external solutions by adjusting the zero current potential to the liquid junction potential. Only cells showing an adequate voltage and space clamp (12) were used.

Experiments were performed at room temperature (21°C–23°C). Data values were each expressed as the mean ± S.E.M., and n represents the number of cells examined. Statistical significance was evaluated with Student’s t-test. P<0.01 was considered significant.
Results

Characterization of TTX-R Na⁺ current in small DRG neurons

TTX-R Na⁺ current is composed of two distinct components, I_{SNS} and I_{NaN}, in small DRG neurons (12). Although the kinetic properties of I_{SNS} and I_{NaN} have already been shown in a number of reports, it appears that the actual values of the kinetic parameters are not consistent, possibly as a result of different experimental conditions, for example, distinctive ionic compositions of the internal and external solutions or different pulse protocols employed to measure kinetic parameters. Therefore, we tried to summarize here some of the fundamental kinetic parameters measured under the same experimental condition for I_{SNS} and I_{NaN}. Typical examples of I_{SNS} and I_{NaN} are shown in Fig. 1 together with the respective current-voltage (I-V) curves. I_{SNS} was activated at about −40 mV, whereas the activation threshold for I_{NaN} was much more negative (about −60 mV). Note the strikingly different time courses of activation and inactivation (compare A-2 and C in Fig. 1).

Segregation of I_{SNS} and I_{NaN}

We isolated TTX-R Na⁺ current from total Na⁺ currents by adding 200 nM TTX to the external solution. The lack of selective agonists or antagonists for TTX-R Na⁺ current precludes the pharmacological approach to separate the two components of TTX-R Na⁺ current. Alternately, isolated I_{NaN} could be obtained from KO DRG neurons in the presence of 200 nM TTX (6, 12, 13). Although it was not possible to segregate I_{SNS} from heterogeneous TTX-R Na⁺ current, it was possible to determine whether or not the cell under recording expresses I_{NaN}. As shown in Fig. 2, I_{NaN} has an activation threshold about 20 mV more negative than the threshold for I_{SNS} (12, 13). Using this criterion, we could obtain isolated I_{SNS} from WT DRG neurons that were devoid of I_{NaN}. The cell shown in Fig. 2A expressed only I_{SNS}.

Fig. 1. Typical examples of two components of tetrodotoxin-resistant (TTX-R) Na⁺ current mediated by NaV_{1.8} (I_{SNS}) and NaV_{1.9} (I_{NaN}). Na⁺ currents were recorded from small (diameter, less than 25 µm) neurons of the mouse dorsal root ganglion (DRG). Since the external solution contained 200 nM tetrodotoxin (TTX) throughout the present experiments, TTX-sensitive Na⁺ currents were totally blocked. A-1: a family of superimposed I_{SNS} recorded from the wild-type (WT) DRG neuron in which I_{NaN} was not detectable (see Fig. 2). Each trace was evoked by a 30-ms test pulse (Vₜ) between −70 and +60 mV in 10-mV steps from a holding potential (Vₜ) of −80 mV. For clarity, only traces evoked by Vₜs of −40 to +10 mV are shown. Voltage labels attached to traces indicate Vₜ in this and subsequent figures. A-2: the traces are also shown with the time scale identical to that employed in panel C. B: the current-voltage (I-V) curve for I_{SNS} shown in panel A. Peak amplitudes of I_{SNS} were plotted against Vₜ. C: a family of superimposed I_{NaN} recorded from the DRG neuron of the NaV_{1.8} knock-out (KO) mouse. Each trace was evoked by a 200 ms Vₜ between −80 and +30 mV in 10-mV steps from a holding potential of −80 mV. For clarity, only traces evoked by Vₜs of −40 to +10 mV are shown. Voltage labels attached to traces indicate Vₜ in this and subsequent figures. D: the I-V curve for I_{NaN} shown in panel C. Since the majority of currents were recorded with a pipette solution containing 3 mM Mg²⁺-ATP in the present study, only traces recorded without Mg²⁺-ATP were specified in subsequent figures.
whereas the cell shown in Fig. 2B expressed both components of TTX-R Na\(^+\) current.

**The effect of PGE\(_2\) on I\(_{\text{SNS}}\)**

Figure 3A shows the effect of PGE\(_2\) (1 \(\mu\)M) on I\(_{\text{SNS}}\) recorded from the WT DRG neuron. In this particular neuron, I\(_{\text{SNS}}\) was not detectable. PGE\(_2\) had no detectable effect on the peak amplitude of I\(_{\text{SNS}}\). Figure 3B shows quantitative results on the effect of PGE\(_2\) (1 and 10 \(\mu\)M) on I\(_{\text{SNS}}\). Here, two different test pulses (V\(_T\)s) were used to detect a possible change in amplitude attributable to the shift of voltage-dependence of activation. There was no significant change in the peak amplitude of I\(_{\text{SNS}}\) or the voltage-dependence of activation for I\(_{\text{SNS}}\), and these results were confirmed by the I-V curves shown in Fig. 4. The possibility that the endogenous PGE\(_2\) attenuated the action of exogenously applied PGE\(_2\) may be excluded since the preparation we used was a dispersed single cell that was continuously superfused by an external solution.

**The effect of PGE\(_2\) on I\(_{\text{NaN}}\)**

We examined the effect of PGE\(_2\) on isolated I\(_{\text{NaN}}\) using KO DRG neurons (Fig. 5). We have shown that the peak amplitude of I\(_{\text{NaN}}\) increases dramatically during whole-cell recording as if inactive or silent Na\(_V\)\(_{1.9}\) channels have been ‘kindled’, when the pipette solution did not contain an ATP compound (12). This phenomenon (kindling of I\(_{\text{NaN}}\)) was effectively suppressed by using a pipette solution containing 3 mM Mg\(^{2+}\)-ATP (12). The amplitude of I\(_{\text{NaN}}\) was measured at two V\(_T\)s as in the case of I\(_{\text{SNS}}\) to check the shift of voltage-dependence of activation. PGE\(_2\) had no detectable effect on the amplitude of I\(_{\text{NaN}}\) or the voltage-dependence of activation.
PGE₂ and TTX-R Na⁺ Current in DRG

activation (Fig. 5A). Figure 5B shows quantitative results on the effect of PGE₂ (1 and 10 µM) on IₕNa. Likewise, PGE₂ had no detectable effect on the I-V curve for IₕNa (Fig. 5: C and D).

The effect of PGE₂ on TTX-R Na⁺ current recorded under nystatin-perforated patch clamp

Some intracellular ingredient that is vital to the action of PGE₂ might have been lost during whole-cell recording. However, the reports that showed the PGE₂-induced upregulation of TTX-R Na⁺ current (15, 16) were based on experiments performed under conventional whole-cell patch clamp. Therefore, it is unlikely that the ineffectiveness of PGE₂ on TTX-R Na⁺ current shown above was due to some detrimental condition inherent to whole-cell patch clamp. This possibility was further excluded by our experiments shown in Fig. 6 where the recordings were performed under the nystatin-perforated whole-cell patch clamp (28). PGE₂ had no detectable effect on either IₛNS (A) or IₕNa (B) even under the nystatin-perforated recordings.

The ‘kindling’ of IₕNa

As has been described, the peak amplitude of IₕNa increases dramatically during whole-cell recording, when the pipette solution did not contain an ATP compound (12). A typical example of this phenomenon is shown in Fig. 7. During recording, the peak amplitude gradually increased, eventually to about 18 times the initial value, and then declined towards the original amplitude. Total duration of this slow and dramatic increase-decrease of the peak amplitude took nearly 20 min. Therefore, there may be a possibility that a sporadic upregulation of IₕNa (kindling) occurred during recordings of heterogeneous TTX-R Na⁺ current, and
consequently, the kindled $I_{\text{NaN}}$ contributed to the up-regulation of heterogeneous TTX-R Na$^+$ current observed during PGE$_2$ treatment.

Figure 8 shows the modulation of the time course of heterogeneous TTX-R Na$^+$ current by the kindling of $I_{\text{NaN}}$ in the WT DRG neuron. Here, the pipette solution again did not contain Mg$^{2+}$-ATP, every 1 min from the KO DRG neuron after establishment of whole-cell clamp condition. Traces are aligned sequentially. Arabic numerals attached to traces represent a time (min) after establishment of whole-cell clamp condition. Note the aberrant increase of the peak amplitude of the current.

Fig. 7. Explosive upregulation (‘kindling’) of $I_{\text{NaN}}$ during whole-cell recording. We recorded $I_{\text{NaN}}$ using a pipette that did not contain Mg$^{2+}$-ATP, every 1 min from the KO DRG neuron after establishment of whole-cell clamp condition. Traces are aligned sequentially. Arabic numerals attached to traces represent a time (min) after establishment of whole-cell clamp condition. Note the aberrant increase of the peak amplitude of the current.

there was no sign of the $I_{\text{NaN}}$ activation despite the fact that this voltage is sufficiently positive to induce $I_{\text{NaN}}$ (see Fig. 2). In addition, the current in response to $V_T$ to 0 mV was completely inactivated within 10 ms. This inactivation time course was apparently much faster than that for $I_{\text{NaN}}$ shown in Fig. 1C. When TTX-R Na$^+$ current was recorded 5 min later, the current was apparently a mixture of $I_{\text{SNS}}$ and $I_{\text{NaN}}$. As shown in Fig. 8B, the voltage-dependence of the activation of the current at 5 min was strikingly different from that at 1 min. The corresponding traces at $-60$ to $-30$ mV are shown superimposed in Fig. 8C to compare the response pattern. It is obvious that the current at 5 min was composed of high-threshold $I_{\text{SNS}}$ and low-threshold $I_{\text{NaN}}$.

On the basis of results shown in Fig. 8, we re-examined the effect of PGE$_2$ in the absence of ATP in the pipette solution. As shown in the inset diagram of Fig. 9, the I-V curve was measured in the WT DRG neuron before and during application of PGE$_2$ (upward arrows). In the I-V curve measured immediately preceding the treatment with PGE$_2$ (IV-2 in Fig. 9), the amplitudes of the current already started to increase. As shown in Fig. 9B, the I-V curve measured 10 min after the establishment of whole-cell clamp condition (IV-3) had much larger amplitude and lower activation threshold than the control I-V curve (IV-1). These observations, together with results in Fig. 8, indicate that the changes in peak amplitude and kinetics were due to the kindling of $I_{\text{NaN}}$, but not due to the effect of PGE$_2$. It should be noted that, the even in the cell which was initially devoid of $I_{\text{NaN}}, I_{\text{NaN}}$ could be often observed later temporarily during continuous recording.
Upregulation of the capsaicin-induced inward current by PGE\textsubscript{2}

There may be a possibility that our experimental condition includes some unidentified fatal defect in producing the effect of PGE\textsubscript{2}. We finally examined this possibility by checking the effect of PGE\textsubscript{2} on the capsaicin-induced inward current, since this current is also known to be upregulated by PGE\textsubscript{2} (29). As shown in Fig. 10, the effect of PGE\textsubscript{2} on the amplitude of the current evoked by capsaicin (100 nM) was examined under the identical experimental situation as that used for TTX-R Na\textsuperscript{+} current. The capsaicin-induced current was dramatically augmented by the prior treatment with PGE\textsubscript{2} (1 \(\mu\)M). This observation was reproducible in 7 out of 10 cells examined.

Discussion

In our previous reports, we could successfully separate the two components of TTX-R Na\textsuperscript{+} current, I\textsubscript{SNS} and I\textsubscript{NaN}, in mouse DRG neurons on the basis of kinetic characterization of these components (12, 13). Consequently, the effect of PGE\textsubscript{2} on the identified component of TTX-R Na\textsuperscript{+} current could be examined in isolation. I\textsubscript{SNS} was recorded by selecting cells that were devoid of I\textsubscript{NaN} (see Fig. 2), while I\textsubscript{NaN} was recorded by using genetically modified mice in which the Na\textsubscript{v}1.8 gene was knocked out. In addition, supplementation of the pipette solution with Mg\textsuperscript{2+}-ATP enabled us to record the stable TTX-R Na\textsuperscript{+} current during an extended period of recording preventing I\textsubscript{NaN} from the kindling (Fig. 7). Although an ATP compound is not necessarily added to the pipette solution in recordings of Na\textsuperscript{+} currents unlike the recordings of Ca\textsuperscript{2+} currents, our results indicate that supplementation of the pipette solution with an ATP compound is vital at least for recordings of TTX-R Na\textsuperscript{+} current.

The effect of PGE\textsubscript{2} on I\textsubscript{SNS}

Unexpectedly, PGE\textsubscript{2} had no detectable effect on either component of TTX-R Na\textsuperscript{+} current contrary to reports that showed upregulation by PGE\textsubscript{2} (15, 16, 23). We used 1 and 10 \(\mu\)M for the concentration of PGE\textsubscript{2}. The concentration of 1 \(\mu\)M was comparable to that employed in other reports and this concentration was supramaximal to evoke the response (15, 16). Furthermore, even the concentration of 10 \(\mu\)M was not effective in our study. The possibility that there was some undesirable
The action of PGE$_2$ on TTX-R Na$^+$ current has been shown to be mediated by a cAMP-protein kinase A-dependent cascade (15). In addition, it has been reported that the effect of PGE$_2$ on TTX-R Na$^+$ current observed under the cell-attached patch configuration was more pronounced and lasted longer than that recorded under whole-cell configuration (16). These findings suggest that modulation of TTX-R Na$^+$ current may involve a diffusible intracellular second messenger. Therefore, conventional whole-cell patch clamp may not be suitable for detection of the action of PGE$_2$ since the diffusible ingredient may be washed out of the cell during whole-cell recording. For avoid this possibility, we also used a nystatin-perforated patch that is likely to protect against washout of the diffusible intracellular ingredients.

**Fig. 9.** An increase in the peak amplitude of TTX-R Na$^+$ current accompanying the treatment of PGE$_2$ was due to the ‘kindling’ of I$_{NaK}$. Inset illustrates the experimental protocol. Four I-V curves (IV-1 – IV-4) were measured 3, 5, 10, and 14 min after the establishment of the whole-cell clamp condition. PGE$_2$ (1 µM) was applied at the period indicated by the bar. A: families of superimposed I$_{NaK}$ were recorded from the WT DRG neuron in response to $V_T$ from $-70$ to $60$ mV from $V_H$ of $-80$ mV through a pipette that did not contain Mg$^{2+}$-ATP. Traces evoked by several $V_T$ values were not shown for clarity. B: I-V curves for recordings shown in panel A. Open circle, IV-1; open square, IV-2; filled circle, IV-3; filled triangle, IV-4.

**Fig. 10.** PGE$_2$ increased the amplitude of capsaicin-induced inward current. Membrane currents at $V_H$ of $-80$ mV were recorded from WT DRG neurons. After several control responses to capsaicin (100 nM) of the constant amplitude were obtained, the effect of PGE$_2$ (1 µM) on the capsaicin-induced current was examined. The trace includes the two consecutive capsaicin-induced currents evoked before and during an application of PGE$_2$. Capsaicin was applied at the filled boxes. PGE$_2$ was applied at a period indicated by the open bar.
However, we could not detect the upregulation of TTX-R Na\textsuperscript{+} current even with this approach (Fig. 6).

At present, we have no idea to explain the discrepancy between our results and those reported by other investigators. The papers that reported the upregulation of TTX-R Na\textsuperscript{+} current by PGE\textsubscript{2} were most probably performed on the heterogeneous TTX-R Na\textsuperscript{+} current comprising I\textsubscript{SNS} and I\textsubscript{NaN}. In this respect, one possible explanation for the discrepancy might be that in earlier reports, the kindling of I\textsubscript{NaN} (a sporadic and aberrant increase of the amplitude of I\textsubscript{SNS}, see Fig. 7) occurred somehow or other, and consequently, the kindling of I\textsubscript{SNS} was misunderstood as the pharmacological effect of PGE\textsubscript{2} when the PGE\textsubscript{2} treatment happened to coincide temporally with the time course of kindling of I\textsubscript{NaN} (see Figs. 8 and 9). Actually, the experiment by England et al. (15) was performed in the absence of an ATP compound in the pipette solution. However, the above possibility cannot explain the result by Gold et al. (16) that was obtained in the presence of 2 mM Mg\textsuperscript{2+} ATP in the pipette solution.

**The effect of PGE\textsubscript{2} on I\textsubscript{NaN}**

The expression of I\textsubscript{SNS} is restricted to the subpopulation of primary afferent neurons with the small cell-body diameter, as in the case of I\textsubscript{NN} (3, 5–9, 11, 12). However, the biophysical properties of the I\textsubscript{SNS} appear to markedly differ from those of I\textsubscript{NN} (i.e., I\textsubscript{NaN} having a more hyperpolarized activation threshold, much slower activation and inactivation kinetics, and more depolarized steady-state inactivation curve; see ref. 12). Therefore, it may be suggested that the role of Na\textsubscript{V}1.9 in pathological pain sensation is distinct from that of Na\textsubscript{V}1.8. Then, an important question that remains to be answered is whether or not I\textsubscript{NaN} is influenced by PGE\textsubscript{2}. Our present results showed that PGE\textsubscript{2} had no detectable effect on I\textsubscript{NaN} (Figs. 5 and 6B).

Coggeshall et al. (3) carried out a histochemical study on Na\textsubscript{V}1.8 and Na\textsubscript{V}1.9 channel proteins in axons of normal and complete Freund’s adjuvant (CFA)-inflamed rat. There were a massive increase in the proportion of Na\textsubscript{V}1.8-labeled myelinated axons and a smaller but still significant increase in the Na\textsubscript{V}1.8-labeled unmyelinated axons in inflamed animals. In contrast, the proportion of Na\textsubscript{V}1.9-labeled unmyelinated axons significantly decreased with no change in the proportion of labeled myelinated axons following inflammation. These histochemical data indicate that Na\textsubscript{V}1.8 and Na\textsubscript{V}1.9 subunits are differentially regulated during inflammation. Our results on I\textsubscript{NaN} are consistent with the above histochemical evidence that Na\textsubscript{V}1.9 may not contribute to peripheral sensitization.

In conclusion, although it is highly likely that the TTX-R Na\textsubscript{V}1.8 subunit plays an important role in PGE\textsubscript{2}-induced sensitization of primary afferent neurons that causes inflammatory hyperalgesia (3, 21), a serious question was raised as to the above compelling hypothesis on the role of TTX-R Na\textsuperscript{+} current. Although we do not exclude the possibility that TTX-R Na\textsuperscript{+} channels play an important role in the PGE\textsubscript{2}-induced sensitization, our data suggest that an augmentation of TTX-R Na\textsuperscript{+} current immediately after the PGE\textsubscript{2} treatment may not be a principal event. In view of our totally negative results, further study is apparently required in an attempt to understand more precisely the mechanistic basis for peripheral sensitization of primary afferent neurons.

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