Enhanced excitability and suppression of A-type K⁺ current of pancreas-specific afferent neurons in a rat model of chronic pancreatitis

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Abstract

PAIN is a cardinal feature of chronic pancreatitis (CP) and remains the most important clinical challenge in these patients (5, 34). Despite much speculation, little is known about the underlying mechanisms responsible for this symptom. By definition, CP is an inflammatory condition and is therefore expected to produce significant functional changes in the nociceptive system serving this organ, as has been observed in other forms of visceral inflammation such as cystitis, gastric ulcers, and ileitis (4, 21, 29, 40). However, until now, it has been difficult to demonstrate this in CP, in large part due to the lack of an appropriate animal model. We (35) have recently developed a novel and robust model of pain behavior and sensitization after the induction of CP with an intraductal injection of trinitrobenzene sulfonic acid (TNBS) in the rat. Three weeks after TNBS treatment, a robust chronic inflammation in the pancreas is seen, which is very similar to changes observed in human CP (9). Rats with TNBS-induced CP also exhibit hypersensitivity including pancreatic hyperalgesia and referred somatic allodynia and hyperalgesia, with the latter responding to conventional analgesics (35). These findings support the construct, face, and predictive validity of this model, rendering it suitable for studying the cellular and molecular responses of nociceptors in CP.

Voltage-gated Na⁺ channels and voltage-gated K⁺ (Kᵥ) channels play a fundamental role in controlling neuronal excitability. Both the upregulation of Na⁺ currents and suppression of Kᵥ currents appear to contribute to peripheral sensitization (2, 3, 29, 40). As part of an ongoing investigation, we first focused on Kᵥ currents of dorsal root ganglion (DRG) neurons innervating the pancreas. A multitude of Kᵥ channels are expressed by nociceptor neurons and have been characterized mainly by their biophysical properties with little molecular correlation. Kᵥ currents are important modulators of spike frequency and activation thresholds (24, 29, 40, 41). Two important Kᵥ currents are the transient A-type Kᵥ current (IᵥA) and the sustained delayed rectifier Kᵥ current (IᵥK) (1, 8, 10, 20). Recent studies in the guinea pig ileitis (29) and rat gastric ulcers (4) have shown that experimentally induced inflammation of the gastrointestinal tract enhanced the excitability of organ-specific sensory neurons, but the changes in voltage-gated ion channels were inconsistent. TNBS-induced ileitis greatly reduced both IᵥK and IᵥA density in DRG neurons innervating the ileal wall (29). The acidic acid-induced gastric ulcers reduced IᵥA density in DRG neurons innervating the stomach, whereas the sustained IᵥK density was not altered (4). Taken together, it is more likely that changes in ion channel function in DRG neurons are organ/disease specific.

In this study, we investigated changes in the membrane properties and excitability of pancreas-specific DRG neurons in response to chronic inflammation. Furthermore, we specifically examined changes in K⁺ channel conductance in these cells, a critical factor in determining the responsiveness of neurons to stimulation. Our findings indicate that CP results in increased spontaneous and evoked excitability of these neurons and that this is associated with a decrease in K⁺ channel currents of the IᵥA type. Our study therefore provides the first mechanistic insight into painful CP from a neurobiological perspective.
perspective and identifies a potential molecular target for therapy.

**MATERIALS AND METHODS**

**Cell labeling and induction of chronic pancreatitis.** Experiments were performed on adult male Sprague-Dawley rats (120–150 g). Care and handling of these animals were approved by the Institutional Animal Care and Use Committee at the University of Texas Medical Branch and were in accordance with the guidelines of the International Association for the Study of Pain. Cell labeling and the induction of CP were performed as previously described (22, 35). The tracer was injected before a pancreatic duct infusion of TNBS or vehicle. In brief, animals were anesthetized with ketamine (80 mg/kg ip) plus xylazine (5–10 mg/kg ip). The abdomen was opened by a midline laparotomy, and the pancreas was exposed. The lipid soluble tracer was injected before a pancreatic duct infusion of TNBS or control vehicle injection, rats were killed by cervical dislocation, followed by removal of DRG neurons from adult control and TNBS-treated rats has been previously described (22, 35). The tracer was injected before a pancreatic duct infusion of TNBS or control vehicle injection, rats were killed by cervical dislocation, followed by removal of DRG neurons from adult control and TNBS-treated rats has been previously described (22, 35).

**biliopancreatic duct and secured with sutures.** TNBS (0.5 ml of 2% solution in 10% ethanol in PBS; pH 7.4) or vehicle was infused into the duct over a period of 2–5 min at a pressure of 50 mmHg. After 30 min, the needle and tubing were removed, the hole in the duodenum was sutured, and the vascular clamp was then removed, which restored the bile flow. The pancreas was gently swabbed before the abdomen was closed. Animals were returned to their housing and given free access to drinking water and standard food pellets.

**Dissociation of DRG neurons and patch-clamp recording.** Isolation of DRG neurons from adult control and TNBS-treated rats has been previously described (38, 39). Briefly, 21–26 days after TNBS or vehicle injection, rats were killed by cervical dislocation, followed by decapitation. DRGs (Tu2,3) were then bilaterally dissected out and transferred to an ice-cold, oxygenated fresh dissecting solution, which contained (in mM) 130 NaCl, 5 KCl, 2 KH2PO4, 1.5 CaCl2, 6 MgSO4, 10 glucose, and 10 HEPES, pH 7.2 (osmolarity: 305 mosM). After removal of the connective tissue, ganglia were transferred to 5 ml of dissecting solution containing collagenase D (1.8–2.0 mg/ml, Roche; Indianapolis, IN) and trypsin (1.2 mg/ml, Sigma; St. Louis, MO) and incubated for 1.5 h at 34.5°C. DRGs were then taken from the enzyme solution, washed, and transferred to 2 ml of the dissecting solution containing DNase (0.5 mg/ml, Sigma). A single-cell suspension was subsequently obtained by repeated trituration through flame-polished glass pipettes. Cells were plated onto acid-cleaned glass coverslips. Coverslips containing adherent DRG cells were put in a small recording chamber (0.5 ml volume) and attached to the stage of an inverting microscope (Olympus) fitted for both fluorescence and bright-field microscopy. DI-labeled neurons were identified by their fluorescence under the fluorescent microscope. For the patch-clamp recording experiments, cells were continuously superfused (1.5 ml/min) at room temperature with normal external solution containing (in mM) 130 NaCl, 5 KCl, 2 KH2PO4, 2.5 CaCl2, 1 MgCl2, 10 HEPES, and 10 glucose, with pH adjusted to 7.4 with NaOH (osmolality: 295–300 mosM). Recording pipettes were pulled from borosilicate glass tubing using a horizontal puller (P-97, Sutter Instruments) and typically had a resistance of 1.5–2.5 MΩ when filled with normal external solution. For perforated patch recording, 3 µl of a 50 mg/ml stock solution of amphotericin B (Calbiochem; La Jolla, CA) in DMSO (Sigma) were added to 0.5 ml of pipette solution to yield a final concentration of 200 µg/ml. The pipette tip was initially filled with amphotericin B-free solution, which contained (in mM) 100 KmeSO3, 40 KCl, and 10 HEPES, with pH 7.25 adjusted with KOH (290 mosM). The pipette was then back filled with the amphotericin B/pipette solution before being used immediately to obtain a gigahm seal. Tip potentials were zeroed before membrane-pipette seals were formed. Perforation of the membrane patch, as revealed by the appearance of slow capacitance transients, occurred within 5–25 min, and recordings were only made when access resistance fell to <15 MΩ. The voltage was clamped at −60 mV by a Dagan 3911 patch-clamp amplifier (Dagan; Minneapolis, MN). Capacitive transients were corrected using capacitive cancellation circuitry on the amplifier that yielded the whole cell capacitance and access resistance. Up to 90% of the series resistance was compensated electronically. Considering the peak outward current amplitudes of <10 nA, the estimated voltage errors from the uncompensated series resistance would be <10 mV. The leak currents at −60 mV were always ≤20 pA and were not corrected. The currents were filtered at 2–5 kHz and sampled at 50 or 100 µs/point. Whole cell current and voltage were recorded with a Dagan 3911 patch-clamp amplifier; and data were acquired and stored on a Dell computer for later analysis using pCLAMP 9.2 (Axon Instruments; Sunnyvale, CA).

**Isolation of Kv currents.** To record Kv currents, Na+ in control external solution was replaced with equimolar choline and the Ca2+ concentration was reduced to 0.03 mM to suppress Ca2+ currents and to prevent Ca2+ channels becoming Na+ conducting (7). The reduced external Ca2+ would also be expected to suppress Ca2+-activated K+ current. The following two kinetically distinct Kv currents were isolated by the biophysical analysis and pharmacological approaches described in previous studies (1, 8, 10, 20, 29): IA and IK. IA and IK were separated biophysically by manipulating the holding potentials. The total outward currents (Iout) were recorded in response to voltage steps from −100 to +30 mV in 5-mV increments with duration of 400 ms. IK was isolated when the membrane potential was held at −50 mV. Subtraction of IK from Iout represented IA. To control for changes in cell size, the current density was measured by dividing the current amplitude by whole cell membrane capacitance (pA/pF), which was obtained by reading the value for whole cell input capacitance cancellation directly from the patch-clamp amplifier.

To study IA and IK channel conductance, activation curves were generated by voltage pulses in 5-mV steps from −80 to +30 mV. To determine the voltage dependence of the steady-state inactivation of IA, two-pulse voltage protocols as previously reported were employed in this experiment. Residual IA was measured after short conditioning pulses (1-s duration), which allowed inactivation of only rapidly inactivating IA. To ensure that IA was not significantly contaminated by slow inactivation of the delayed rectifier, we analyzed kinetic data on current decay. The decay phase of the currents was well fitted with a single exponential. The fitting correlation coefficient was much closer to 1 and the SD was much smaller compared with two or three exponentials. The time constant of inactivating IA was 145 ± 11 ms (n = 5) at a test pulse of 30 mV, which is close to previous data of inactivating IA of neurons innervating the rat urinary bladder (40) and guinea pig intestine (29). This excludes the possibility of contamination of our results by slow inactivation of the delayed rectifier.

**Data analysis.** The membrane conductance (G) at each command potential (Vcom) was determined by dividing the measured membrane current (I) by the driving force as follows: \[ G = \frac{I(V_{m} - E_{k})}{E_{k}} \] where EK is the equilibrium K+ potential and was calculated to be −76 mV (external [K+] = 7 mM and internal [K+] = 140 mM). Activation data (G–V curve) were fitted by the following modified Boltzmann equation: \[ G = \frac{G_{\text{max}}}{1 + \left[ 1 + \left( \frac{V - V_{1/2}}{k} \right)^{m} \right]^{-m}} \] where Gmax is the fitted maximal conductance, V1/2 is the membrane potential for half-activation, and k is the slope factor. Steady-state inactivation of IA was fitted with the following negative Boltzmann equation: \[ I_{\text{II}} = \frac{\text{amplifier}}{\text{G425 CHRONIC PANCREATITIS AND DRG NEURON MEMBRANE PROPERTIES G425}} \]
1/[1 + \exp(-(V_{1/2} - V_m)/k)], where \(I_{\text{max}}\) is maximal current. Inactivation data were plotted as \(I_{\text{max}}/V\) versus the prepulse voltage used to generate the inactivation curves. No neuron with a resting membrane potential (RP) more depolarized than \(-40\) mV was included in the data analysis. All data are expressed as means ± SE. Statistical significance was determined by Student’s t-test or Fisher’s exact test, as appropriate.

RESULTS

Alteration in spontaneous activities and RPs. To determine the effect of TNBS treatment on the excitability of pancreas-specific DRG neurons, RPs of DRG neurons from control and TNBS-treated rats were first studied. DRG neurons were acutely dissociated; labeled pancreas-specific neurons were identified under fluorescent microscopy and studied using current-clamp techniques. In control rats, RPs of neurons recorded were very stable with \(<3\)-mV changes within 2 min of observation (Fig. 1A), and no spontaneous firing was seen (Table 1). In TNBS-treated rats, however, RPs of neurons (\(n = 7\)) recorded showed marked fluctuations, ranging from 5 to 15 mV within a 2-min observation period (Fig. 1B). In addition, four neurons displayed spontaneous firings (Fig. 1C). The spontaneous firing frequency varied between neurons and ranged from 0.5 to 6 Hz (average: 3.4 Hz, \(n = 4\)). The percentage of neurons with spontaneous oscillations (including spontaneous firings) was significantly higher than that of the control group (Table 1; \(P < 0.01\)). We also compared RPs of DRG neurons from both groups after excluding electrically unstable (fluctuation >5 mV) neurons. As a group, pancreas-specific DRG neurons from animals with CP were significantly more depolarized at rest than controls (Fig. 1D and Table 1; \(P < 0.005\)). These data suggest that a subset of pancreas-specific neurons is spontaneously active in the setting of CP.

Reduction of current threshold and increase in cell firing frequency. To further study the excitability of pancreas-specific DRG neurons in CP, we examined the current threshold (rheobase) and the pattern of firing in response to depolarizing current stimulation. The rheobase is the minimal current injection to induce one action potential (AP). In this study, the average rheobase of DRG neurons from TNBS-treated animals was markedly lower than that of controls (Fig. 2, A, B, top, and C, and Table 2; \(P < 0.01\)). In addition, the numbers of APs in response to a current stimulation (2 × rheobase) were also examined. The numbers of evoked APs were different in these two groups: \(1.2 ± 0.3\) APs/100 ms (range 1–2, \(n = 16\)) and \(2.0 ± 0.3\) APs/100 ms (range 1–3, \(n = 16\)) in the control group compared with \(3.1 ± 0.8\) APs/100 ms (range 2–5, \(n = 16\)) and \(5.8 ± 0.8\) APs/100 ms (range 2–7, \(n = 16\)) (Fig. 2E, top, and F, and Table 2; \(P < 0.02\)) in the CP group.

Table 1. Properties of spontaneous activities of pancreas-specific DRG neurons from control and TNBS-treated rats

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Quiescent</th>
<th>Oscillations/Firings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>22 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>TNBS</td>
<td>37</td>
<td>26 (70.3)</td>
<td>11* (29.7)</td>
</tr>
</tbody>
</table>

Values are numbers of neurons recorded, with percentages shown in parentheses. DRG, dorsal root ganglion; TNBS, trinitrobenzene sulfonic acid. *\(P < 0.01\) compared with the control (by Fisher’s exact test).

To examine whether changes in rheobase and firing frequency of neurons from TNBS-treated rats could be explained simply by the observed differences in the RP, in further experiments, we adjusted the RPs to \(-58\) mV (the average level of neurons from control rats) by injecting hyperpolarizing current into the cells recorded from TNBS-treated rats. After membrane potential adjustment, the rheobase for evoking an AP of neurons from the TNBS-treated group was not significantly altered (Fig. 2B, bottom) and was still markedly lower than that in the control group (TNBS correction: \(0.20 ± 0.05\) nA, \(n = 5\); control: \(0.61 ± 0.11\) nA, \(n = 20\)) (\(P < 0.05\)). The numbers of repetitive firings evoked by two times rheobase stimulation after membrane potential adjustment remained significantly higher than those in the control group (Fig. 2E, bottom; TNBS correction: \(3.0 ± 0.8\) APs/100 ms, \(n = 5\); control: \(1.2 ± 0.3\) APs/100 ms, \(n = 16\)) (\(P < 0.05\)).

Several additional electrical properties were also examined, with the results shown in Table 2. Notably, both AP duration (at 0 mV) and membrane input resistance in TNBS-treated rats were significantly greater than those in controls (\(P < 0.05\)). On the other hand, AP threshold and amplitude were not significantly changed after TNBS treatment (Table 2). TNBS treatment did not alter the cell size distribution of pancreas-specific neurons (Fig. 3 and Table 2). In both the control (\(n = 117\)) and TNBS-treated groups (\(n = 111\)), pancreas-specific DRG neurons were mainly medium-sized neurons (\(>20\) and \(<35\) \(\mu\)m).
with small-size neurons (<20 μm) and large-size neurons (>35 μm) making a smaller contribution. To simplify our analysis, only small- and medium-sized cells were included in this study.

Suppression of I_A density. Because changes in spike frequency and activation thresholds suggest an alteration in Kv channels (24, 29, 40, 41), we next performed perforated patch-clamp recordings to examine these currents under voltage-clamp conditions. Na^+ in the control external solution was replaced with equimolar choline and the Ca^{2+} concentration was reduced to 0.03 mM. A depolarization step from -100 to -30 mV in 5-mV increments with a duration of 400 ms activated all Kv channels (I_total; Fig. 4A). The peak current-voltage (I-V) curves are shown in Fig. 4D. TNBS treatment significantly reduced I_total density in DiI-labeled neurons compared with the control (control: 114.0 ± 4.5 pA/pF, n = 6; TNBS: 88.1 ± 5.5 pA/pF, n = 9, P < 0.05; Fig. 4G). Because there were two main types of Kv currents (I_A and I_K) described in nociceptive DRG neurons, we then isolated these two

Table 2. Membrane characteristics of pancreas-specific DRG neurons in control and TNBS-induced chronic pancreatitis rats

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TNBS</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter, μm</td>
<td>32.2±1.0 (35)</td>
<td>32.9±0.8 (46)</td>
<td>NS</td>
</tr>
<tr>
<td>Resting potential, mV</td>
<td>-57.9±1.1 (22)</td>
<td>-46.8±1.3 (26)</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Current threshold, nA</td>
<td>0.6±0.1 (20)</td>
<td>0.2±0.03 (20)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AP threshold, mV</td>
<td>-20.0±1.0 (24)</td>
<td>-20.6±1.2 (22)</td>
<td>NS</td>
</tr>
<tr>
<td>Number of AP, 100-ms</td>
<td>1.2±0.3 (16)</td>
<td>3.1±0.8 (16)</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>depolarization</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AP amplitude, mV</td>
<td>88.4±3.9 (20)</td>
<td>87.8±2.2 (19)</td>
<td>NS</td>
</tr>
<tr>
<td>AP duration, ms</td>
<td>2.8±0.4 (15)</td>
<td>4.6±1.0 (20)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Input resistance, MΩ</td>
<td>118.6±16.2 (12)</td>
<td>196.5±15.9 (18)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Values are mean ± SEM, with sample size in parenthesis. AP, action potential. Numbers of APs were recorded by injection of 2 times rheobase pulse. NS, no significance. Values are means ± SE, with sample sizes shown in parentheses. Numbers of action potentials (APs) were recorded by injection of a 2× rheobase pulse. NS, not significant. P values were determined by a Student’s t-test.

![Fig. 2. Reduction in rheobase and increase in firing frequency. Representative traces of action potentials were induced by 100-ms depolarizing current pulses injected through the patch pipette at rheobase (A and B) and two times rheobase (D and E) in labeled neurons from control and TNBS-treated rats under current-clamp conditions.](http://ajpgi.physiology.org/)
Fig. 4. TNBS-induced chronic pancreatitis (CP) significantly reduced the A-type K+ current (IA) density. Currents were measured at different holding potentials. A: for total voltage-gated K+ (Kv) current, the membrane potential was held at −100 mV and voltage steps were from −50 to +30 mV with 5-mV increments and 400-ms duration. B: for sustained K+ current, the membrane potential was held at −50 mV and the voltage steps were the same as above. C: currents generated by these two protocols were subtracted to produce IA. The peak currents of total current (I_{total}; D), outward rectifier K+ current (I_K; E) and IA (F) versus voltages (I-V) were plotted from the above representative cell. IA amplitude was measured as the peak of the transient component, and IK amplitude was measured at the ending point of 400-ms voltage steps. G-F: bar graphs showing the mean peak IA density compared with controls (H; P > 0.05); however, the IA density was significantly reduced from 72.8 ± 6.0 pA/pF (n = 6) in the control group to 48.0 ± 6.7 pA/pF (n = 9) in the TNBS group (I; *P < 0.05). Current subtraction revealed that CP did not alter the IK density compared with controls (H; P > 0.05); the IA density was significantly reduced from 72.8 ± 6.0 pA/pF (n = 6) in the control group to 48.0 ± 6.7 pA/pF (n = 9) in the TNBS group (I; *P < 0.05).

kinetically different K_+ currents by manipulating the holding membrane potential. A depolarization step from −50 to +30 mV in 5-mV increments with duration of 400 ms activated most of the sustained K_+ channels but not A-type K_+ channels (Fig. 4, B and E). Subtraction of IK from I_{total} yields IA (Fig. 4, C and F). IA was further confirmed by the application of the A-type channel blocker 4-aminopyridine (4-AP; 5 mM, data not shown) and the reversal potential, which was determined for each individual current from its instantaneous tail current. Because the reversal potential of IA and IK was close (−71 mV) to the predicted reversal potential for K+; it is assumed that these currents are carried by potassium ions. The peak I-V curves are shown in Fig. 4, E and F. In this experiment, IA density was remarkably reduced in animals with CP (control: 72.8 ± 6.0 pA/pF, n = 6; TNBS: 48.0 ± 6.7 pA/pF, n = 9, P < 0.05; Fig. 4I), whereas IK density was not significantly changed (control: 51.7 ± 12.0 pA/pF, n = 6; TNBS: 50.4 ± 7.1 pA/pF, n = 9, P > 0.05; Fig. 4H).

Leftward shift in the inactivation curve of IA Because a reduction in the peak current density of IA was evident, we then analyzed the voltage dependence of activation and inactivation of electrophysiologically isolated IA channels. Activation-voltage relationships were constructed from I-V curves of neurons from control and TNBS-treated rats. Currents at various test pulses were divided by the driving force for K+ (with −76 mV as the value for E_K and the resulting conductance was expressed as a percentage of that achieved at +30 mV for each condition (Fig. 5A). The G-V relationships were fitted with a Boltzmann equation to derive values for V_{1/2} and k. The G-V curve obtained from the control group had a V_{1/2} of −19.8 ± 3.1 mV and k of 12.7 ± 1.7 (n = 7; Fig. 5, C and D). TNBS treatment did not significantly change the V_{1/2} (−15.7 ± 1.8 mV) or k (14.6 ± 2.4) of the G-V curve (n = 8; Fig. 5, A, C, and D). To determine whether the reduction in IA involved a shift of the steady-state inactivation curve, a two-pulse voltage protocol was employed as described above. Inactivation curves were obtained by plotting IA during the test pulse against the membrane potential during the conditioning pulse. IA was normalized to I_{max} during the test pulse to 0 mV after a conditioning pulse to −160 mV (Fig. 5B). Data were fitted with the negative Boltzmann function. The I-V curve of steady-state inactivation obtained from the control group had a V_{1/2} of −73.6 ± 2.9 mV and k of 4.9 ± 0.4 (n = 3; Fig. 5, C and D). In contrast, the V_{1/2} and k of the I-V curve of inactivation from the TNBS-treated group were −94.5 ± 5.9 mV (n = 4; Fig. 5C) and 5.2 ± 0.6, respectively (n = 4; Fig. 5D). Thus TNBS treatment led to a ∼20-mV negative shift in the V_{1/2} of the steady-state inactivation curve (P < 0.05); as such, at −60 mV, the IA in pancreas-specific DRG neurons from the TNBS-treated group was almost negligible. In contrast, ∼20% of I_{max} could be available for activation in the control group. Therefore, this shift in the steady-state inactivation curve can account for the enhanced excitability of pancreas-specific DRG neurons in animals with CP.

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DISCUSSION

Inflammation of an organ is characteristically accompanied by peripheral sensitization, a phenomenon in which sensory neurons respond in an abnormal and exaggerated manner to stimulation. The effect or processes by which sensitization is mediated by several different classes of ion channels such as transient receptor potential and voltage-dependent Na$^+$, K$^+$, and Ca$^{2+}$ channels. The purpose of our study was to examine the effects of CP on the electrophysiological properties of pancreatic sensory neurons and to provide insight into potential ionic mechanisms. To do this, we took advantage of our previously validated rat model for studying pain behavior in CP induced by intraductal TNBS. This model is accompanied by pancreatic allodynia and hyperalgesia as well as increases in neuropeptide expression and release by sensory neurons, providing strong evidence for peripheral sensitization (35). In this study, we provide the first direct evidence for hypersensitivity of pancreas-specific primary sensory neurons in the setting of CP. This conclusion is based on several findings shown in Figs. 1 and 2. First, pancreatic neurons from animals with CP displayed marked fluctuation of RP, spontaneous discharges, and more depolarized RPs than controls (Fig. 1 and Table 1), indicating that these neurons are spontaneously active. Second, these neurons exhibited lower current thresholds for initiating an AP compared with controls. Finally, these neurons had enhanced firing frequencies in response to a standardized stimulation compared with controls (Fig. 2 and Table 2).

Another interesting finding is that TNBS-treatment significantly suppressed $I_A$ density in pancreas-specific DRG neurons (Fig. 4). This is consistent with and an extension of previous reports of models of cystitis, ileitis, and gastric ulcers (4, 21, 26, 29, 40). Therefore, the reduction in $I_A$ density may well contribute to the enhanced excitability of pancreas-specific neurons in our model as well. Possible mechanisms for the reduction of $I_A$ density include somal hypertrophy, downregulation of $I_A$ channel expression, and changes in channel properties. Because the cell diameter was not altered after the TNBS treatment (Fig. 3 and Table 2), the reduction of $I_A$ density cannot be attributed simply to somal hypertrophy of pancreas-specific DRG neurons. Although single-channel properties and $I_A$ channel expression of pancreas-specific neurons after inflammation have yet to be studied, our analysis of the voltage dependence of steady-state inactivation showed that the $I-V$ curve for $I_A$ channels shifted significantly in a hyperpolarized direction in the setting of CP (Fig. 5B). As such, this would make fewer $I_A$ channels available at or near RPs. According to our results, ~20% of $I_A$ channels were available for activation at ~60 mV in the control group compared with only ~3% in the TNBS-treated group. Because there was no significant change of the activation-voltage rela-

![Fig. 5. Steady-state activation and inactivation curves for $I_A$. A: for activation curves, $I_A$ was generated by two voltage pulses in 5-mV increment steps from −100 to 30 mV in one DRG neuron from a control rat and one from a TNBS-treated rat. The reversal membrane potential (V$_{rev}$) in this recording condition was −71 mV. Membrane conductances (G) at different test potentials were measured by dividing the peak $I_A$ by the current driving force (V$_{rev}$ − V$_{test}$), and were normalized to that recorded at 30 mV (G$_{max}$). Data were fitted with the following Boltzmann equation: G/G$_{max}$ = 1/[1 + exp(−(V − V$_{1/2}$)/k)], where V is membrane potential, V$_{1/2}$ is the membrane voltage at which the current was half-maximally activated, and k is the slope factor. TNBS treatment did not change the activation curve compared with the control. V$_{1/2}$ was −19.8 ± 3.1 mV (n = 7) for neurons from control rats and −15.7 ± 1.8 mV (n = 8) for neurons from TNBS-treated rats (C); k was 12.7 ± 1.7 (n = 7) and 14.6 ± 2.4 mV (n = 8) for neurons from control and TNBS-treated rats, respectively (D). B: for steady-state inactivation curves, a long conditional step of various voltages from −160 to 0 mV in 10-mV increment was followed by a testing pulse to +30 mV. These inactivation curves are representative curves of one neuron from a control rat and one neuron from a TNBS-treated rat, respectively. The peak current amplitude was normalized to that recorded at a −160-mV conditional step (I$_{max}$). Data were plotted as a function of conditional step potentials and fitted with the following Boltzmann equation: I/I$_{max}$ = 1/[1 + exp(−(V$_{1/2}$ − V)/k)]. TNBS treatment resulted in a significant leftward shift of the steady-state inactivation curve. V$_{1/2}$ was −73.6 ± 2.9 mV for neurons (n = 3) from the control group and −94.5 ± 5.9 mV for neurons (n = 4) from the TNBS group (C; *P < 0.05); k was 4.9 ± 0.4 and 5.2 ± 0.6 mV for neurons from control (n = 3) and TNBS-treated rats (n = 4), respectively (D; P > 0.05).]
function and expression of Kv channels because a large body of evidence shows that NGF plays an important role in producing sensitization in somatic pain models (37) and in acute and chronic CP (30, 35, 36) and affecting ion channels and membrane properties in afferent sensory neurons and pheochromocytoma-12 cells (18, 27, 42). To better understand the molecular basis of chronic changes in K⁺ currents in inflammatory states, further studies should be performed on the expression of Kv channels, especially the Kv1.4 channel, which may be the dominant IA channel protein in nociceptors (23) and is downregulated in DRGs in animal models (12, 15, 23).

In addition to the effect of IA, the contribution of other K⁺ channels to the enhanced excitability of pancreas-specific neurons has also to be considered. Sustained K⁺ currents have been reported to be altered after TNBS-induced ileitis (29). In this study, TNBS-induced CP had no effect on IK because the IK density was not significantly changed in the TNBS group compared with controls (Fig. 4H). Because IA was obtained by subtracting the current evoked from −50 mV from that evoked from −100 mV, it is possible that IA might be contaminated by IK subject to steady-state inactivation. However, this seems unlikely because IA was defined as that arising between the holding potential and potential minimal just after the capacity transient, 2–4 ms from the beginning of the depolarization steps. This point of measurement for IA was used to minimize contamination with IK. In addition, IA was completely blocked by 4-AP (2 mM, data not shown), whereas IK was resistant to 4-AP. Furthermore, the kinetic data on IA decay showed that the time constant of this current was 145 ms at a test potential of 30 mV, which is close to previous data of inactivating IA of neurons innervating the rat stomach (4), rat urinary bladder (40), and guinea pig intestine (29). Therefore, the contamination of IA with IK was minimal in this study. Pancreas-specific neurons from rats with CP also displayed an increased input membrane resistance compared with controls (Table 2). Changes in RP and membrane input resistance may also be mediated by modulation of hyperpolarization-activated cation current (IH; an inwardly rectifying current) or leakage current (IL; an outward resting current) (25). However, the depolarization of RP in our study is unlikely to be due to an increase in IH, as this would be expected to result in a decrease in input resistance. The depolarization of RPs in pancreatic DRG neurons in CP may have resulted from a decrease in IL, resulting in an increase in input membrane resistance. However, in our experiments, when the RPs of DRG neurons from TNBS-treated rats were corrected to the normal level (i.e., −58 mV), the rheobase and cell spike frequency were not significantly changed compared with those before the correction of RP (Fig. 2). Thus, even if there were changes in IL, they had a minimal effect on spike frequency and current threshold.

These changes in membrane properties and Kv currents thus provide a clear neurobiological explanation for the previously reported behavioral responses in this model (35) and set the stage for further mechanistic studies on pain in CP. Advances in knowledge in this area have been limited by the lack of a suitable experimental model. We (36) and others (6, 11, 16, 19, 28, 33) have previously reported on nociceptive changes in rat models of acute pancreatitis. Although important, these studies were limited by the fact that there are significant differences between the neurobiology of acute pancreatitis and CP. There has only been one previous report (32) measuring abdominal hypersensitivity in a “chronic” model using dibutyryl in-duce inflammation. However, dibutylin affects tetrodotoxin-resistant Na⁺ currents in sensory neurons in addition to its effect as a general neurotoxin (13, 14, 17, 31), thus seriously confounding the interpretation of the findings. In contrast, the TNBS-induced model of CP is morphologically similar to human CP (construct validity) and also displays face and predictive validity, thus representing a robust experimental approach for the establishment of working paradigms to explain the pathogenesis of pain in CP. Our findings of a lower current threshold and enhanced firing frequencies of pancreas-specific nociceptive DRG neurons (Fig. 2) are similar in some ways to those reported in experimental inflammation of other visceral organs such as the urinary bladder (26, 40), stomach (4), and intestine (21, 29). However, there are also notable differences. Thus no changes in RP were seen in either a mouse model of colitis (induced by TNBS) (2) or a rat model of cystitis (induced by cyclophosphamide) (40). Furthermore, we did not find any changes in neuronal size in our model, unlike the significant increases reported with interstitial cystitis in cats (26), chronic cystitis in rats (40), and TNBS ileitis in guinea pigs (21). Although some of these differences may be species related, our results also suggest that nociceptor changes may be organ and/or disease specific, further attesting to the importance of an appropriate model.

In conclusion, our data demonstrate that TNBS-induced CP is associated with an increased neuronal excitability and suppression of IA in pancreas-specific DRG neurons. Because changes in current threshold and spike frequency all suggest alteration of A-type K⁺ channels, the observed suppression of IA in our study assumes particular significance, potentially identifying for the first time a specific molecular mechanism underlying visceral pain and hypersensitivity in CP. This information and further studies hold the promise for providing new strategies for the treatment of pain in CP.

GRANTS

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REFERENCES


