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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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 alldynia 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 (Iₐ) and the sustained delayed rectifier Kv current (Iₖ₁) (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 that changes in voltage-gated ion channels were inconsistent. TNBS-induced ileitis greatly reduced both Iₐ and Iₖ₁ density in DRG neurons innervating the ileal wall (29). The acetic acid-induced gastric ulcers reduced Iₐ density in DRG neurons innervating the stomach, whereas the sustained Iₖ₁ 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 Iₐ 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 fluorescence dye 1,1'-dioleyl-3,3',3'-tetramethylindocarbocyanine methanesulfonate (DiI; Molecular Probes; Eugene, OR), 25 mg in 0.5 ml methanol, was injected in 2-μl volumes at 8–10 sites on the exposed pancreas. To prevent leakage and possible contamination of adjacent organs with the dye, the needle was left in place for 1 min and each injection site was washed with normal saline after each injection. For the induction of CP, the common bile duct was closed temporarily near the liver with a small vascular clamp. A blunt 28-gauge needle with polyethylene-10 tubing attached was gently inserted into the duodenum and guided through the papilla into the biliopancreatic duct and secured with sutures. TNBS (0.5 ml of 2% solution of TNBS 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.

**Isolation of DRG neurons and patch-clamp recording.** Isolation of DRG neurons from adult control and TNBS-treated rats has been described previously (38, 39). Briefly, 21–26 days after TNBS or vehicle injection, rats were killed by cervical dislocation, followed by decapitation. DRGs (T9–13) were then bilaterally dissected out and described previously (38, 39). With amphotericin B-free solution, which contained (in mM) 100 KmeSO₄, 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 gigaohm 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 amplifiers 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 by a computer equipped with a 16-bit analog to digital converter and 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): I₆ and I₅. I₅ and I₆ were separated biophysically by manipulating the holding potentials. The total outward currents (I₆otal) were recorded in response to voltage steps from −100 to +30 mV in 5-mV increments with duration of 400 ms. I₅ was isolated when the membrane potential was held at −50 mV. Subtraction of I₅ from I₆otal represented I₆. 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 capacitance and access resistance directly from the patch-clamp amplifier.

**Data analysis.** The membrane conductance (G) at each command potential (V_cm) was determined by dividing the measured membrane current (I) by the driving force as follows: \[ G = \frac{I}{(V_{cm} - E_k)} \], where \( E_k \) is the equilibrium \( K^+ \) potential and was calculated to be −76 mV (external \( [K^+] = 7 \text{ mM} \) and internal \( [K^+] = 140 \text{ mM} \)). Activation data (G-V curve) were fitted by the following modified Boltzmann equation: \[ G/G_{max} = 1/[1 + \exp(-(V_{1/2} - V_cm)/k))] \], where \( G_{max} \) is the fitted maximal conductance, \( V_{1/2} \) is the membrane potential for half-activation, and \( k \) is the slope factor. Steady-state inactivation of I₅ was fitted with the following negative Boltzmann equation: \[ I/I_{max} = 1/[1 + \exp(-(V_{1/2} - V_cm)/k))] \].
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 current-clamp techniques. No neuron with a resting membrane potential (RP) more depolarized than $-40\,\text{mV}$ was included in the data analysis. All data are expressed as means $\pm$ SE. Statistical significance was determined by Student’s t-test or Fisher’s exact test, as appropriate.

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>TNBS</th>
<th>Oscillations/Firings</th>
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<tbody>
<tr>
<td>Total</td>
<td>22</td>
<td>37</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Quiescent</td>
<td>22 (100)</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 ganglia; TNBS, trinitrobenzene sulfonic acid. *$P < 0.01$ compared with the control (by Fisher’s exact test).

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 $>5\,\text{mV}$) 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 $\times$ rheobase) were also examined. The numbers of evoked APs were different in these two groups: 1.2 $\pm$ 0.3 APs/100 ms (range 1–2, n = 16; Fig. 2D) in the control group compared with 3.1 $\pm$ 0.8 APs/100 ms (range 2–5, n = 16; Fig. 2, E, top, and F, and Table 2; $P < 0.02$) in the CP group.

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 $-58\,\text{mV}$ (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 $\pm$ 0.05 nA, n = 5; control: 0.61 $\pm$ 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 $\pm$ 0.8 APs/100 ms, n = 5; control: 1.2 $\pm$ 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 $\mu\text{m}$).

Fig. 1. Dorsal root ganglion (DRG) neurons from trinitrobenzene sulfonic acid (TNBS)-treated rats are spontaneously active and rest at more depolarized membrane potentials. A and B: example of resting membrane potential (RP) recorded from DRG neurons from control (Con; A) and TNBS-treated rats (B). The RP was very stable in the control group but exhibited a 5- to 15-mV fluctuation after TNBS treatment. C: spontaneous firings recorded from a neuron from a TNBS-treated rat. D: bar graph showing the average of RP of neurons from control and TNBS-treated rats. TNBS treatment significantly depolarized the RP. *$P < 0.005$. 

\[ 1/[1 + \exp(-(V_{1/2} - V_m)/k)] \] 

where $I_{\text{max}}$ is maximal current. Inactivation data were plotted as $I_{\text{max}}$ versus the prepulse voltage used to generate the inactivation curves. No neuron with a resting membrane potential (RP) more depolarized than $-40\,\text{mV}$ was included in the data analysis. All data are expressed as means $\pm$ SE.
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>
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<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. 3. Cell size distribution of pancreas-specific DRG neurons.** TNBS treatment did not significantly alter the size distribution among DRG neurons compared with controls (small: diameter ≥25 μm, medium: diameter >25 μm and <35 μm, and large: diameter ≥35 μm).
revealed that CP did not alter the G in the TNBS group (*the control group to 48.0 n 7.1 pA/pF, *TNBS group (*for each individual current from its instantaneous tail current. Not shown) and the reversal potential, which was determined as the value for A. The peak currents of total current (I_total; D), outward rectifier K+ current (I_K; E) and I_K (F) versus voltages (I-V) were plotted from the above representative cell. I_K amplitude was measured as the peak of the transient component, and I_K amplitude was measured at the ending point of 400-ms voltage steps. G-I bar graphs showing the mean peak I_total (G), I_K (H), and I_A (I) densities from control and TNBS-treated rats. The current density (in pA/pF) was calculated by dividing the current amplitude by cell membrane capacitance. TNBS-induced CP caused a significant reduction of total Kv current from 114.0 ± 4.5 pA/pF (n = 6) in the control group to 88.1 ± 5.5 pA/pF (n = 9) in the TNBS group (G; *P < 0.05). Current subtraction revealed that CP did not alter the I_K density compared with controls (H; P > 0.05); however, the I_A 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).

**Leftward shift in the inactivation curve of I_A.** Because a reduction in the peak current density of I_A was evident, we then analyzed the voltage dependence of activation and inactivation of electrophysiologically isolated I_A 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 I_A 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 I_A during the test pulse against the membrane potential during the conditioning pulse. I_A was normalized to I_A during the test pulse to 0 mV after a conditioning pulse to −140 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 I_A in pancreas-specific DRG neurons from the TNBS-treated group was almost negligible. In contrast, ~20% of I_A 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.
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 maintained include signal transduction, generation of APs, and neurotransmitter release. These functions are in turn subserved by several different classes of ion channels such as transient receptor potential and voltage-dependent Na 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-
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 inafferent 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 axotomy 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 dibutyltin to induce inflammation. However, dibutyltin 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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