Expression and function of a CP339,818-sensitive K\(^{+}\) current in a subpopulation of putative nociceptive neurons from adult mouse trigeminal ganglia

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Sforna L, D’Adamo MC, Servettini I, Guglielmi L, Pessia M, Franciolini F, Catacuzzeno L. Expression and function of a CP339,818-sensitive K\(^{+}\) current in a subpopulation of putative nociceptive neurons from adult mouse trigeminal ganglia. J Neurophysiol 113: 2653–2665, 2015. First published February 4, 2015; doi:10.1152/jn.00379.2014.—Trigeminal ganglion (TG) neurons are functionally and morphologically heterogeneous, and the molecular basis of this heterogeneity is still not fully understood. Here we describe experiments showing that a subpopulation of neurons expresses a delayed-rectifying K\(^{+}\) current (\(I_{\text{DRK}}\)) with a characteristically high (nanomolar) sensitivity to the dihydropyridine CP339,818 (CP). Although submicromolar CP has previously been shown to selectively block Kv1.3 and Kv1.4 channels, the CP-sensitive \(I_{\text{DRK}}\) found in TG neurons could not be associated with either of these two K\(^{+}\) channels. It could neither be associated with Kv1.2 channels homomeric or heteromERICally associated with the Kv9.2, Kv9.3, or Kv6.4 subunits, whose block by CP, tested using two-electrode voltage-clamp recordings from Xenopus oocytes, resulted in the low micromolar range, nor to the Kv7 subfamily, given the lack of blocking efficacy of 3 \(\mu\)M XE991. Within the group of multiple-firing neurons considered in this study, the CP-sensitive \(I_{\text{DRK}}\) was preferentially expressed in a subpopulation showing several nociceptive markers, such as small membrane capacitance, sensitivity to capsaicin, and slow afterhyperpolarization (AHP); in these neurons the CP-sensitive \(I_{\text{DRK}}\) controls the membrane resting potential, the firing frequency, and the AHP duration. A biophysical study of the CP-sensitive \(I_{\text{DRK}}\) indicated the presence of two kinetically distinct components: a fast deactivating component having a relatively depolarized steady-state inactivation \((I_{\text{DRK}})\) and a slow deactivating component with a more hyperpolarized \(V_{1/2}\) for steady-state inactivation \((I_{\text{DRK}})\).

trigeminal ganglion neurons; voltage-gated K\(^{+}\) currents; CP339,818; nociceptors; afterhyperpolarization

The trigeminal ganglion (TG) contains a heterogeneous population of neurons carrying to the central nervous system a variety of somatosensory information originating from the head, such as touch, warm and cold sensation, spatial position and movement of the head, and pain. Depending on the type of somatosensory information carried, sensory neurons possess different morphological, biochemical, and electrophysiological properties, which are used to infer from in vitro studies the somatosensory function that a neuron possesses in vivo, a procedure most common with nociceptors (Lawson 2002; Catacuzzeno et al. 2014). Several studies have shown that most nociceptors have a low (C-type) conduction velocity, unmyelinated fibers, and relatively small cell bodies (Harper and Lawson 1985); thus a small cell body diameter is usually taken as an indication of a likely nociceptive neuron (Lawson 2002). The expression of peptidergic neurotransmitters such as substance P (SP) and calcitonin gene-related peptide (CGRP) has also been linked to nociceptive function, as both neurotransmitters are expressed in a higher proportion of nociceptive than nonnociceptive sensory neurons (Lawson 2002). A further indication of a neuron’s nociceptive nature is the expression of specific membrane receptors used to detect painful stimuli, such as the cation-selective channel transient potential receptor vanilloid (TRPV1), gated by high temperature, low pH, and capsaicin (Caterina and Julius 2001).

Nociceptive sensory neurons can also be identified on the basis of their specific electrophysiological properties, such as longer action potentials (AP) and afterhyperpolarizations (AHP) and higher AP overshoot than nonnociceptive neurons (Lawson 2002; Fang et al. 2005). Notably, while the higher AP overshoot and the longer AP duration in nociceptive neurons can be explained by the abundant expression of high levels of tetrodotoxin-resistant Na currents and high voltage-gated Ca currents (Blair and Bean 2003), the ionic basis of the longer AHP is still unclear, even though in principle slowly deactivating K\(^{+}\) currents could well serve the purpose. Further investigation on this aspect is needed since a long AHP, generally observed in central and peripheral neurons, contributes to the ability of a neuron to discharge multiple APs during a prolonged depolarization or repetitive stimulation (Cloues and Sather 2003).

Voltage-gated K\(^{+}\) (Kv) channels are ubiquitously expressed in neurons, where they participate in setting their excitability by controlling the resting membrane potential, the AP repolarization time, the repetitive firing frequency, and the amplitude and duration of the fast AHP. Tens of molecularly and functionally different Kv channels exist (Coetzee et al. 1999), and their particular expression pattern in a specific neuron is tuned by its excitability requirements (Pongs 1999). In adult mouse TG neurons we recently observed that the differential expression of specific types of Kv channels determines three distinct firing patterns in response to prolonged depolarizing stimuli (Catacuzzeno et al. 2008). More specifically, the preferential expression of low-threshold dendrotoxin (DTX)-sensitive Kv subunits in the large-size neuronal population prevents a single firing in response to prolonged depolarizations [single-
spiking (SS) neurons]. Conversely, the preferential expression of a fast-inactivating, A-type Kv current confers to a fraction of small-size neurons a characteristic delay preceding the multiple firing response [delayed multiple firing (DMF) neurons]. Finally, a third subpopulation of still small-size TG neurons, lacking DTX-sensitive and A-type K+ currents, expresses a delayed rectifier Kv current (I_{DRK}) that allows them to fire multiple APs in response to prolonged stimulation [multiple firing (MF) neurons]. Unfortunately, the molecular identity of the I_{DRK} expressed in MF neurons is still unknown. Based on the expression of nociceptive markers, such as capsaicin sensitivity and AP and AHP duration, the MF population appears extremely heterogeneous, suggesting that it contains both nociceptive and nonnociceptive neurons (Catacuzzeno et al. 2008; Fioretti et al. 2011). A central question thus remains on whether nociceptive and nonnociceptive MF neurons express different types of K+ currents composing their I_{DRK}.

In this article we describe experiments showing that a subpopulation of MF neurons displays an I_{DRK} with a characteristically high (nanomolar) sensitivity to the dihydroquinine line CP339,818 (CP). Although CP has been shown to block a terminal current of the AHP. Neurons showing several nociceptive markers and found to contain the following (in mM): 130 NaCl, 5 KCl, 0.3 KH2PO4, 4 NaHCO3, 0.3 Na2HPO4, 10 HEPES, and 10 glucose at pH 7.4 with NaOH. After 30 min, the ganglia were diced into small pieces and incubated for 40 min at 37°C in 1 mg/ml collagenase (Type XI-s; Sigma) in HBSS. Individual cells were dissociated by triturating the tissue through fire-polished glass pipettes having a progressively smaller tip diameter. The dissociated cells were then incubated for 10 min at 37°C in 0.5 mg/ml DNase I (Type IV; Sigma) and further dissociated using Pasteur pipettes. After being washed three times with DMEM medium ( GibCO), the cells were cultured in DMEM supplemented with 10% fetal bovine serum, 100 IU/ml penicillin + 100 μg/ml streptomycin, plated on poly-L-lysine-coated 35-mm Petri dishes, and cultured overnight at 37°C in a water-saturated atmosphere with 5% CO2. Neurons were studied 12–24 h after plating. Only neurons without or with short processes were used. All experiments were carried out at room temperature (20–25°C).

Electrophysiological Recordings in TG Neurons

Whole cell, patch-clamp experiments under voltage-clamp and current-clamp configuration were performed with an EPC-10 amplifier (HEKA), using borosilicate pipettes with a resistance of 3–5 MΩ. Seventy percent of the access resistance was actively compensated under voltage-clamp configuration. Only recordings having a residual access resistance <10 MΩ were considered for further analysis. Current and voltage traces were sampled at 25 kHz and low-pass filtered at 3 kHz. Cell capacitance, membrane, and access resistance were measured in voltage-clamp configuration at the beginning of the experiment (after ~2 min from the achievement of the whole cell configuration) using the automatic compensation circuitry of the EPC-10 amplified, at a holding potential of ~60 mV. Assessments of CP sensitivity and cell excitability were performed only in TG cells having a membrane capacitance <25 pF and without evident fast transient K+ currents. These cells have been previously shown to belong to the MF subpopulation. The cell excitability was evaluated in current-clamp mode by applying the following three protocols: 1) continuous recording of 1-min duration with no applied current, to allow the electrical stabilization of the cell at the resting membrane potential; 2) series of brief (1 ms) depolarizing current pulses from the resting potential, to evaluate the AP shape; and 3) series of long (500 ms) depolarizing current pulses from the resting potential, to evaluate the MF properties. Only cells showing a stable resting membrane potential during the application of the three protocols were considered for further analysis. Whole cell experiments were performed using an intracellular solution containing the following (in mM): 135 KCl, 1 MgCl2, 10 EGTA-K 10, 10 HEPS, 4 NaATP, 1 and NaGTP at pH 7.2 with KOH, and an extracellular solution containing the following (in mM): 145 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPS, and 10 glucose at pH 7.4. Perforated patch experiments were performed using an intracellular solution containing the following (in mM): 130 K-glucenate, 20 KCl, 2 MgCl2, and 10 HEPS at pH 7.2 with KOH, and an extracellular solution containing the following (in mM): 145 NaCl, 5 KCl, 2 CaCl2, 1 MgCl2, 10 HEPS, and 10 glucose at pH 7.4 with NaOH.

Electrophysiological Recordings in Xenopus Oocytes

mKv9.1 and mKv9.2 (kindly provided by Michel Lazdunski, Université de Nice Sophia Antipolis, Valbonne, France) were subcloned into pEco vector, and hKv2.1 and hKv6.4 were subcloned into pBF1 vector. Capped mRNAs were synthesized in vitro by using the SP6 mMESSAGE mMACHINE kit (Ambion, TX) and microinjected into Xenopus oocytes. Xenopus laevis were deeply anesthetized with an aerated solution containing 3-aminobenzoic acid ethyl ester methanesulfonate salt (5 mM) and sodium bicarbonate (60 mM), pH 7.3. To further reduce their suffering, Xenopus laevis underwent no more than two surgeries, separated by at least 3 wk. Stage V–VI Xenopus oocytes were isolated, injected with 50 nl mRNAs, and stored at 16°C in fresh ND96 medium containing the following (in mM): 96 NaCl, 2 KCl, 1 MgCl2, 1.8 CaCl2, 5 HEPS, and 50 μg/ml gentamicin (Sigma). Procedures involving Xenopus laevis and their care were in accordance with the regulations of the Italian Animal Welfare Act, the local Authority Veterinary Service, and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The minimal number of animals was used. Two-electrode voltage-clamp (TEVC) recordings were performed from oocytes at room temperature (20–25°C) and 1–8 days after injection (D’Adamo et al. 1999). A GeneClamp 500 amplifier (Axon Instruments, Foster City, CA) interfaced to a PC computer with an ITC-16 interface (Instrutech, Longmont, CO). Microelectrodes were filled with 3 M KCl and had resistances of 0.1–0.5 MΩ. The extracellular solution contained the following (in mM): 96 NaCl, 2 KCl, 1 MgCl2, 1.8 CaCl2, and 5 HEPS at pH 7.4. Currents were evoked by voltage commands from a holding potential of ~80 mV as described in the figure legends. Recordings were filtered at 2 kHz and acquired at 5 kHz with Pulse software. Leak and capacitative currents were subtracted using a P/4 protocol.

Chemicals and Toxins

Capsaicin (Sigma) and CP339,818 (Tocris Bioscience) were dissolved in DMSO stock solutions at concentrations of 1 and 10 mM.
respectively. The final concentration of DMSO in the perfusing solution never overcame 0.1%. We verified that this concentration of DMSO did not have significant effects on TG neuron excitability and K⁺ currents. Dendrotoxin I (DTX-I; Alomone Labs) and margatoxin (MgTX; Alomone Labs) were dissolved in water stock solutions at concentrations of 100 μM.

Data Analysis

The time course of the current, as well as kinetic and steady-state parameters, were fitted with the indicated equations by using the Simplex algorithm incorporated in Microcal Origin v 4.1. The χ² statistic was used as an indicator of the quality of the fit (Dempster 1993). Unless otherwise indicated, χ² values for the fits of the experimental data shown in RESULTS correspond to levels of significance at P < 0.05. Results are expressed as means ± SE. Statistical differences between means were analyzed using the t-test that does not assume equal variances. Where appropriate the significance level of P for the difference between mean values is given. In multiple comparisons, the t-test was performed when the one-way ANOVA analysis detected a significant difference (P < 0.05). The concentration-response relationship for the block by CP of homomeric and heretomeric Kv2.1 channels expressed in oocytes was fitted with the single-site Hill relationship (Catacuzzeno et al. 2008; Fig. 1C) fitted by the following sum of two Hill relationships

\[
\text{residual current} = \frac{A_1}{1 + \left(\frac{[CP]}{K_{d1}}\right)^n} + \frac{1 - A_1}{1 + \left(\frac{[CP]}{K_{d2}}\right)^n} \tag{1}
\]

where \(K_{d1}, n_1, \) and \(K_{d2}, n_2\) represent the dissociation constants and Hill coefficients for the first and second site, respectively, while \(A_1\) is the fractional contribution of the first blocking site.

The concentration-response relationships for the block by CP of homomeric and heretomeric Kv2.1 channels expressed in Xenopus oocytes were fitted with the single-site Hill relationship

\[
\text{residual current} = \frac{1}{1 + \left(\frac{[CP]}{K_d}\right)^n} \tag{2}
\]

where \(K_d\) and \(n\) represent the dissociation constant and Hill coefficient, respectively.

The steady-state inactivation of the slow CP-sensitive component was estimated by assessing the slow tail current amplitude at varying depolarization-activated outward current at 0 mV of applied potential. The steadystate inactivation of the slow CP-sensitive component was estimated by assessing the slow tail current amplitude at varying prepulse potential and fitting the data with the following Boltzmann relationship

\[
\text{normalized amplitude} = \frac{1}{1 + e^{-\frac{V - V_{1/2}}{k}}} \tag{3}
\]

The activation/deactivation time constants as a function of the applied potential for the fast and slow CP-sensitive components were best fitted with the following relationships:

\[
\text{Activation } \tau_{\text{DRKf}} = 0.005 + 0.111e^{-0.00418} \tag{4}
\]

\[
\text{Deactivation } \tau_{\text{DRKd}} = 0.0474 + 0.16v \tag{5}
\]

\[
\text{Activation } \tau_{\text{DRKs}} = 0.051 + 0.102e^{-((v - 0.021)/0.009)^2} \tag{6}
\]

\[
\text{and deactivation } \tau_{\text{DRKd}} = 0.172 + 0.716e^{(v/0.0203)} \text{ for } v < -0.01 \tag{7}
\]

RESULTS

Expression of a CP-Sensitive \(I_{\text{DRK}}\) in Adult Mouse MF Neurons

All the experiments presented in this study have been performed on TG neurons belonging to the MF subpopulation, characterized by a small soma size (membrane capacitance <25 pF), an outward current with no inactivation for depolarizations <0 mV, and the ability to respond with a MF to a long depolarizing stimulus under current-clamp configuration (Catacuzzeno et al. 2008; Fig. 1, A and B). Whole cell recordings under control conditions, performed in MF neurons of small soma size (membrane capacitance <25 pF), showed a depolarization-activated outward current with a low degree of inactivation (Catacuzzeno et al. 2008; Fig. 1, A and B). These neurons could be subgrouped in two different subpopulations on the basis of the sensitivity of their \(I_{\text{DRK}}\) to low concentrations of CP (Fig. 1, A and B). More specifically, in 24 out of 34 (70%) neurons the \(I_{\text{DRK}}\) was markedly inhibited (ca. 80%) by CP concentrations of 1–3 μM (CP-sensitive neurons; Fig. 1B), while in the remaining neurons these CP concentrations were essentially ineffective (CP-insensitive neurons; Fig. 1A), and much higher concentrations were needed to significantly inhibit the current (data not shown). At these higher CP concentrations (i.e., ≥10 μM), the residual \(I_{\text{DRK}}\) remaining upon applications of low CP concentrations in

Fig. 1. A and B: current trace families obtained from a CP339,818 (CP)–insensitive (A) and a CP-sensitive (B) neuron by applying depolarizing steps from −40 to +60 mV (in 20-mV step) in control conditions and following the application of 3 μM CP. Inset: plot showing the percentage of CP-sensitive and CP-insensitive multiple firing (MF) neurons found during the study. A neuron was defined as CP-sensitive if 1–3 μM CP blocked ≥15% of its depolarization-activated outward current at 0 mV of applied potential. C: plot showing the fractional residual current as a function of CP concentration applied to the bathing solution, assessed from CP-sensitive MF neurons at −20 mV of applied potential (n = 3–6 for each experimental point). The continuous line represents the best fit of the experimental data with a double Hill relationship (Eq. 1). The best fit parameters were as follows: \(a_1 = 0.743, K_{d1} = 0.032\) μM, \(n_1 = 1.66, K_{d2} = 5.84\) μM, \(n_2 = 2.67\).
CP-sensitive neurons was also completely suppressed. This is illustrated in Fig. 1C, showing a concentration-response relationship for CP-sensitive neurons displaying two very distinct components, with ~80% of the current being blocked with high affinity (estimated $K_d$ of 32 nM), and the remaining current being blocked by nearly 200-fold higher concentrations of CP (estimated $K_d$ of 5.8 μM).

To clarify the molecular nature of the CP-sensitive current, we used a pharmacological approach designed to assess the sensitivity of the CP-sensitive component to a given pharmacological agent. The experimental protocol used is illustrated in Fig. 2A and here briefly described. The outward $K^+$ current was continuously monitored by stimulating the neuron every 10 s with a pulse to 0 mV preceded by a 1.5-s long pulse at −110 mV. At the beginning of the experiment, the pharmacological agent was applied to determine its effect on the total K current (including both the CP-sensitive and the CP-insensitive components). After washout, 1 μM CP was applied to determine in the same cell the amount of the CP-sensitive component, and finally the pharmacological agent was applied in the continuous presence of CP to assess its effect on the CP-insensitive component. This allowed to determine the fractional residual CP-sensitive current in presence of any given pharmacological agent using the relationship:

$$\text{fractional residual current} = \frac{(I_0 - I_1) - (I_2 - I_3)}{(I_0 - I_2)}$$

where $I_0$, $I_1$, $I_2$, and $I_3$ are the currents recorded under control condition, in the presence of the pharmacological agent to be

![Fig. 2. A: time course of the K current evoked in a CP-sensitive neuron in response to a depolarizing step to 0 mV preceded by a 1.5-s long prepulse to −110 mV (from a holding potential of −60 mV) showing the effects of bath application of 10 mM tetraethylammonium (TEA), 1 μM CP, and 1 μM CP + 10 mM TEA (as indicated). Also indicated is the relationship used to assess the fractional CP-sensitive residual current in presence of 10 mM TEA. Inset: current traces obtained at the time points indicated in main panel. B: bar plot showing the fractional residual current of MF CP-sensitive neurons in the presence of either 100 nM dendrotoxin-I (DTX-I); 50 nM MgTX; 0.1, 1, or 10 mM TEA; 1 or 10 mM 4-aminopyridine (4-AP); or 3 μM XE991, assessed from experiments similar to that shown in A. ***$P < 0.001$, t-test; **$P < 0.01$, t-test. C: current traces recorded from a Kv2.1-expressing Xenopus oocytes in response to depolarizing steps to +60 mV, from a $V_H$ of −80 mV, in control conditions and in presence of various concentrations of CP (indicated). D: plot showing the fractional residual current as a function of CP concentration applied to the bathing solution, assessed from Kv2.1 (squares, $n = 11$), Kv2.1 + Kv6.4 (circles, $n = 5$), Kv2.1 + Kv9.1 (open triangles, $n = 3$), and Kv2.1 + Kv9.2 (closed triangles, $n = 3$) expressing oocytes. The continuous lines represent the best fits of the experimental data with the Hill relationship Eq. 2. The best fit parameters are as follows: Kv2.1: $K_d = 4.82$ μM, $n = 1.69$; Kv2.1 + Kv6.4: $K_d = 3.85$ μM, $n = 1.61$; Kv2.1 + Kv9.1: $K_d = 3.92$ μM, $n = 1.58$; Kv2.1 + Kv9.2: $K_d = 5.65$ μM, $n = 1.58$.}
tested, in the presence of CP, and in the presence of both CP and the pharmacological agent, respectively.

CP has been shown to block only Kv1.3 and Kv1.4 channels at submicromolar concentrations (K_i: 0.23 and 0.3 μM, respectively), while being much less effective (K_i: >10 μM) on other Kv channels (Kv1.1, Kv1.2, Kv1.5 and Kv1.6, Kv3.1–3.3, and Kv4.2 channels; Nguyen et al. 1996). We therefore verified whether the CP-sensitive I_{DRK} in TG neurons was carried by Kv1.3 or Kv1.4 channels by assessing whether it had the pharmacological profile typical of these channels. We first probed margatoxin (MgTX; 50 nM), a potent blocker of monomeric and heteromeric Kv1.3-containing channels (Garcia-Calvo et al. 1993; Vicente et al. 2006), and found that it was ineffective on the CP-sensitive I_{DRK}, suggesting that Kv1.3 subunits do not contribute to this current (Fig. 2B). As for Kv1.4 homomeric channels, they display such a fast rate of inactivation as to make them unlikely to sustain the CP-sensitive I_{DRK} that does not show any fast inactivation (cf. Fig. 1B, bottom). However, the inactivation rate of Kv1.4 homomeric channels can be reduced when Kv1.4 subunits are coexpressed with other noninactivating Kv1 subunits (Po et al. 1993) and be completely prevented when coexpressed with Kv1.6, displaying an N-type inactivation prevention domain (Al-Sabi et al. 2011). As these subunits are often found to form heteromeric channels in association with Kv1.4 in neurons (Rhodes et al. 1995; Sheng et al. 1992; Cooper et al. 1998; Imbrici et al. 2006, 2011), we assessed the effect of the Kv1.1, Kv1.2, and Kv1.6 inhibitor DTX-I. As shown in Fig. 2B, 100 nM DTX-I was found completely ineffective on the CP-sensitive I_{DRK}, also excluding this possibility.

We also assessed the effects of the wide-range K channel blockers tetaethylammonium (TEA) and 4-aminoypyridine (4-AP) on the CP-sensitive current and found that while the current was sensitive to TEA in the millimolar range, it was not significantly blocked by 1 mM 4-AP, and 10 mM 4-AP only blocked ~20% of the CP-sensitive current (Fig. 2B). Finally, we tested the M-current-specific inhibitor XE991 since M currents carried by KCNQ2/3 subunits have been described in dorsal root ganglion (DRG) and TG neurons (Passmore et al. 2003; Ooi et al. 2013; Linley et al. 2012) and have a sensitivity to TEA and 4-AP similar to the CP-sensitive current. We probed 3 μM XE991 and found that it did not significantly affect the CP-sensitive current (Fig. 2B). On this basis we can exclude that the CP-sensitive current is sustained by KCNQ2/3 subunits, whose IC_{50} for XE991 is much lower than 1 μM.

As the inhibitory efficacy of CP has never been tested on Kv2 channels, we expressed the Kv2.1 subunit in Xenopus oocytes but found that this channel has a low affinity for CP (Fig. 2, C and D). Since several Kv9 and Kv6 subunits have been shown to be expressed in sensory neurons (Tsantoulas et al. 2012; Bocksteins et al. 2009), where they could potentially be associated with Kv2 channels and change their pharmacological sensitivity, we also tested the sensitivity to CP of Kv2.1/Kv9.2, Kv2.1/Kv9.1, and Kv2.1/Kv6.4 heteromeric channels and found all of them essentially insensitive to block by CP (Fig. 2D). Therefore, the molecular nature of the CP-sensitive I_{DRK} in mouse MF neurons remains at the moment undetermined.

The CP-sensitive I_{DRK} Includes Two Biophysically Distinct Components

In a fraction of CP-sensitive neurons we applied a series of voltage protocols in control conditions and following 3 μM CP to assess the kinetic and steady-state activation and inactivation properties of the CP-sensitive I_{DRK}. Figure 3A shows current families obtained by applying depolarizing voltage steps from −60 to +40 mV preceded by a 1.5-s conditioning step to either −40 or −110 mV in control conditions and following the application of 3 μM CP. It is clear that the CP-sensitive I_{DRK} (lower current families obtained as point-by-point subtraction of the respective traces above) shows substantially different current density and voltage-dependence depending on the pre-conditioning potential used in the stimulation protocol. More specifically, CP-sensitive I_{DRK} had higher amplitude and began to activate at more hyperpolarized potentials, i.e., with preconditioning prepulse at −110 mV compared with −40 mV (Fig. 3B). These results suggest that the CP-sensitive I_{DRK} includes multiple components differing in their steady-state activation and inactivation properties. To better investigate this aspect, we analyzed the tail currents of the CP-sensitive I_{DRK} obtained upon repolarization to −55 mV following depolarizing pulses to 0 mV preceded by either −40- or −110-mV preconditioning pulses (Fig. 3C). Tail currents obtained following a −40-mV preconditioning pulse deactivated with a monoexponential time course showing a relatively fast time constant [Fig. 3C, right trace; mean τ_{−40} = 53.5 ± 10.0 ms; n = 6]. By contrast, tail currents obtained from the same cells following a −110-mV preconditioning pulse deactivated with a biexponential time course (Fig. 3C, left trace). The fast time constant was very similar to that obtained with the −40-mV preconditioning pulse [τ_{−110} = 46.5 ± 3.5 ms; n = 6; P > 0.05 vs. τ_{−40}], while the slow time constant had an approximately five times higher value [τ_{−110} = 257 ± 19 ms; n = 6; Fig. 3C, bottom right]. In addition, the best fit initial current density of the fast deactivating component was significantly higher for −110-mV compared with −40-mV conditioning pulses (1.84 ± 0.16 vs. 1.30 ± 0.24 nA; P < 0.05; Fig. 3C, bottom left). The simplest interpretation of these results is that the CP-sensitive current is made up of two biophysically distinct components: a fast deactivating component having a relatively depolarized steady-state inactivation (hereafter called I_{DRKf}) and a slow deactivating component with a more hyperpolarized V_{1/2} for steady-state inactivation (hereon called I_{DRKs}).
CP-sensitive $I_{DRK}$ activation properties. The steady-state and kinetic activation of $I_{DRK}$ were studied from CP-sensitive currents evoked from a $-40$-mV preconditioning step, a condition that inactivates virtually all the $I_{DRK}$ (cf. Fig. 3E). Tail current amplitudes immediately after repolarization to $-55$ mV, normalized to the maximal value obtained in the same cell, were taken as a measure of the activation degree of $I_{DRK}$ at the preceding depolarizing step (Fig. 4A). Mean normalized tail current amplitudes as a function of voltage could be well fitted by a Boltzmann relationship with $V_{1/2}$ of $5.2 \text{ mV}$ and a $k$ of $10.1 \text{ mV}$ (Fig. 4B). The activation time course of $I_{DRK}$ at all voltages tested could be well fitted by a monoeXponential function (Fig. 4C) with a time constant displaying a strong voltage dependence (Fig. 4D).

The voltage-dependence of $I_{DRK}$ steady-state activation was assessed from CP-sensitive currents evoked with depolarizing steps preceded by a $-110$-mV preconditioning step and by measuring the tail current amplitudes at 200 ms from the beginning of the repolarizing step, a time at which the $I_{DRK}$ is fully deactivated (cf. Figs. 3 and 4A). As shown in Fig. 4, E and F, the mean normalized tail current amplitude as a function of voltage could be well fitted by a Boltzmann relationship having a $V_{1/2}$ of $-6.4 \text{ mV}$ and a $k$ of $14.9 \text{ mV}$, suggesting that $I_{DRK}$ activates at voltages substantially more hyperpolarized than those required for $I_{DRK}$ activation (cf. with dashed curve in Fig. 4F, representing the voltage-dependence of $I_{DRK}$ activation).

The activation kinetics of $I_{DRK}$ was studied using the following procedure. $I_{DRK}$ evoked at varying depolarizing steps preceded by either $-40$ or $-110$ mV were recorded from the same neuron (cf. Fig. 3A). The current trace evoked with a $-40$-mV preconing pulse was then fitted with a monoeXponential function to assess the $I_{DRK}$ activation time con-
A CP-SENSITIVE K CURRENT IN TRIGEMINAL GANGLION NEURONS

Fig. 4. A: CP-sensitive tail currents obtained from a MF neuron in response to a series of depolarizing steps from −40 to +60 mV, preceded by a 1.5-s preconditioning step to −40 mV. B: plot of the mean normalized tail current amplitude as a function of the preceding membrane potential, obtained from 5 neurons as that shown in A. The continuous line represents the best fit of the experimental data with a Boltzmann equation having $V_{1/2} = 5.2$ mV and $k = 10$ mV. C: CP-sensitive current trace obtained in response to a depolarizing step to +20 mV of applied potential preceded by a 1.5-s preconditioning step to −40 mV. The continuous line represents the best fit of the current trace with the function $I = (1 - e^{-t/\tau})^n$. D: plot of the mean best fit parameter $n$ as a function of the applied potential, obtained from 5 neurons as that shown in C. The continuous line represents the best fit of the experimental data with Eq. 4, where $a_1 = 5$ ms, $a_2 = 111$ ms, and $k = 14.5$ mV. Inset: plot of the mean best fit parameter $n$ as a function of voltage. E: CP-sensitive tail currents obtained from a MF neuron in response to a series of depolarizing steps from −40 to +60, preceded by a 1.5-s preconditioning step to −110 mV. F: plot of the mean normalized tail current amplitude, measured 200 ms from the beginning of the repolarizing step, as a function of the preceding membrane potential, obtained from 5 neurons as that shown in A. The continuous line represents the best fit of the experimental data with a Boltzmann equation having $V_{1/2} = −6.4$ mV and $k = 15$ mV. G: CP-sensitive current traces obtained in response to a depolarizing step to 20 mV of applied potential preceded by a 1.5-s preconditioning step to either −40 or −110 mV. The continuous lines represent the best fit of the current traces with either the function $I = (1 - e^{-t/\tau})^n$ (conditioning step at −40 mV) or the function $I = (1 - e^{-t/\tau})^n + (1 - e^{-t/\tau})^p$ (conditioning step at −110 mV). H: plot of the mean best fit parameter $n$ as a function of the applied voltage, obtained from 5 neurons as that shown in G. The continuous line represents the best fit of the experimental data with Eq. 6. Inset: plot of the mean best fit parameter $n$ as a function of voltage.

stant (cf. Fig. 4C). To assess the activation properties of $I_{\text{DRK}}^s$, the current trace obtained with a −110-mV preconditioning pulse was then fitted using the equation $a_1 (1 - e^{-t/\tau})^n + a_2 (1 - e^{-t/\tau})^p$, where $a_1$ and $a_2$ values were those obtained from the analysis previously performed on currents evoked from a −40-mV preconditioning pulse. As shown in Fig. 3, G and H, this analysis gave a molecularity of ∼1 for the $I_{\text{DRK}}^s$, with an activation time constant strongly voltage dependent and generally higher than that obtained for $I_{\text{DRK}}$.

The CP-Sensitive $I_{\text{DRK}}$ Is Preferentially Expressed in Putative Nociceptive MF Neurons

We next assessed whether the CP-sensitive $I_{\text{DRK}}$ was preferentially expressed in putative nociceptors within the MF TG subpopulation. To this end we performed experiments aimed at verifying whether an association existed between the neuron sensitivity to CP and the presence of recognized nociceptive markers. We first looked at the soma size of CP-sensitive and CP-insensitive neurons and found that CP-sensitive neurons had an electrical membrane capacitance significantly smaller than CP-insensitive neurons (Fig. 5C), suggesting that the CP-sensitive $I_{\text{DRK}}$ is preferentially expressed in small-size MF neurons. Since nociceptive neurons display several features of the AP shape that distinguish them from nonnociceptive neurons (cf. Introduction), we also compared the shape of the AP evoked by brief (1 ms) depolarizing pulses in CP-sensitive and CP-insensitive neurons. As shown in Table 1, of the several parameters describing the AP shape, a significant difference was only found for the AHP duration, which resulted markedly higher in CP-sensitive neurons (34.9 ± 5.6 vs. 17.2 ± 2.6 ms; $P < 0.05$; Table 1 and Fig. 5, D–F). We finally tested the responsiveness of CP-sensitive and CP-insensitive neurons to the TRPV1 agonist capsaicin, known to evoke a cationic current selectively in nociceptive sensory neurons (Caterina and Julius 2001). As shown in Fig. 5, G–I, while most CP-sensitive neurons (92%) responded to capsaicin application with a significant inward current at −60 mV of applied potential, only few CP-insensitive neurons were capsaicin responsive (30%), suggesting a strong association between the expression of the CP-sensitive current and that of TRPV1 channels. In conclusion, our data indicate the preferential expression of the CP-sensitive $I_{\text{DRK}}$ in MF neurons showing TRPV1 channel expression, a relatively small soma size, and a relatively long duration AHP, in other words, in putative MF nociceptive neurons.

The CP-Sensitive $I_{\text{DRK}}$ Contributes to the Excitability of MF Neurons

We then turned to verify whether the CP-sensitive $I_{\text{DRK}}$ contributes to the excitability properties of MF neurons. To this end, we performed current-clamp experiments, applying a

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500-ms depolarizing stimuli of various amplitude, under control conditions and following bath application of 1 or 3 μM CP. We found that CP had a significant effect on the excitability of 30 out of 37 (81%) MF neurons tested, in accordance with our previous results that only ~70% of MF neurons express a CP-sensitive \( \varphi_{BRK} \) (cf. Fig. 1). The main effect of CP on neuron excitability consisted in a significant increase in the number of APs evoked during the 500 ms depolarizing step (Fig. 6; \( P < 0.05 \)). Parallel effects were a small but significant depolarization of the resting membrane potential and a substantial decrease in the depolarizing current needed to evoke an active response (Fig. 6, A–C; \( P < 0.05 \)). Since a change in the resting membrane potential may change the availability of a number of neuronal ion channels, the effect of CP on the MF properties could be secondary to the depolarizing effect. To verify this possibility, in three neurons, after the application of 1 μM CP, we reset the resting potential to levels similar to those observed under control conditions. After this maneuver, an effect of 1 μM CP on the MF frequency was still evident (data not shown), suggesting that the CP-sensitive K current has an effect on the MF frequency independent of its ability to depolarize the resting membrane potential.

To verify whether the increased firing frequency in the presence of CP is the result of a shorter and faster HAP (Cloues et al. 2001), we evoked APs using 1-ms depolarizing pulses and assessed the effect of CP on the AHP duration and decay rate (Fig. 6D). As shown in Fig. 6, E and F, CP significantly shortened the duration of the AHP and increased the depolarization rate after the AHP peak, suggesting that these changes may be responsible for the observed effect of CP on the firing frequency.

\( \text{Ca}^{2+} \)-activated K channels are often found to significantly contribute to the AHP in many types of neurons, and there are several lines of evidence for the presence of these channels at least in a subpopulation of DRG nociceptors (Wu et al. 2013; Zhang et al. 2010; Li et al. 2007; Gruss et al. 2001). We therefore verified on CP-sensitive neurons the

Table 1. AP parameters of CP-sensitive and CP-insensitive neurons

<table>
<thead>
<tr>
<th>Parameter</th>
<th>CP-Sensitive Neurons (n)</th>
<th>CP-Insensitive Neurons (n)</th>
<th>( P ) (CP-Sensitive vs. CP-Insensitive)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_r ), mV</td>
<td>-43.9 ± 2.2(11)</td>
<td>-46.5 ± 4.6 (6)</td>
<td>0.5655</td>
</tr>
<tr>
<td>( \text{AP}_{\text{amp}} ), mV</td>
<td>43.7 ± 1.1 (11)</td>
<td>48.9 ± 3.7 (5)</td>
<td>0.2029</td>
</tr>
<tr>
<td>( \text{AP}_{\text{r}} ), ms</td>
<td>6.6 ± 1.1 (11)</td>
<td>14.8 ± 1.8 (6)</td>
<td>0.0980</td>
</tr>
<tr>
<td>( \text{AHP}_{\text{amp}} ), mV</td>
<td>18.4 ± 1.5 (11)</td>
<td>14.8 ± 1.8 (6)</td>
<td>0.0955</td>
</tr>
<tr>
<td>( \text{AHP}_{\text{dur}} ), ms</td>
<td>34.9 ± 5.6 (11)</td>
<td>17.2 ± 2.6 (6)</td>
<td>0.0424*</td>
</tr>
<tr>
<td>( E_{\text{a}} ), V/s</td>
<td>101.5 ± 27.9 (11)</td>
<td>150.2 ± 38.1 (6)</td>
<td>0.3170</td>
</tr>
<tr>
<td>( t_{\text{a}} ), ms</td>
<td>-37.8 ± 6.5 (11)</td>
<td>-50.8 ± 7.5 (6)</td>
<td>0.3170</td>
</tr>
<tr>
<td>( t_{\text{g}} ), V/s</td>
<td>-27.3 ± 2.1 (11)</td>
<td>-40.4 ± 8.0 (5)</td>
<td>0.0509</td>
</tr>
<tr>
<td>( t_{\text{r}} ), ms</td>
<td>5.1 ± 1.0 (10)</td>
<td>3.08 ± 0.7 (5)</td>
<td>0.2546</td>
</tr>
<tr>
<td>( \text{rheo}\text{S}, \text{pA/pF}</td>
<td>290 ± 75.6 (11)</td>
<td>366.7 ± 76.1 (6)</td>
<td>0.0727</td>
</tr>
<tr>
<td>( \text{rheoL}, \text{pA/pF}</td>
<td>22.7 ± 9.5 (11)</td>
<td>93.3 ± 28.9 (6)</td>
<td>0.0825</td>
</tr>
</tbody>
</table>

Values are means ± SE. \( V_r \), resting membrane potential; \( \text{AP}_{\text{amp}} \), action potential amplitude; \( \text{AP}_{\text{r}} \), AP repolarization time; \( \text{AHP}_{\text{amp}} \), AHP amplitude; \( \text{AHP}_{\text{dur}} \), AHP duration. *Significant difference.
contribution of Ca\textsuperscript{2+}-activated K channels to the AHP evoked after a single AP, using iberiotoxin (IbTx) and apamin (Apa), inhibitors of large-conductance (BK) and small-conductance (SK) Ca\textsuperscript{2+}-activated K channels, respectively. As shown in Fig. 6, G and I, neither 100 nM IbTx nor 500 nM Apa were able to affect the AHP shape and amplitude, suggesting that, at least under our recording conditions, Ca\textsuperscript{2+}-activated K channels are not contributing to the excitability of MF neurons. Similar results were also obtained upon combined application of 100 nM IbTx and 500 nM Apa, using the perforated-patch configuration that preserves the endogenous cell buffering power (Fig. 6, H and I). This suggests that the negative results obtained in whole cell configuration were not caused by the high Ca-buffering power of our pipette solution, containing 10 mM EGTA.
DISCUSSION

The main observations of our study are as follows: 1) a subpopulation of mouse MF TG neurons expresses a voltage-gated K+ current sensitive to nanomolar concentrations of the dihydroquinoline CP; 2) the pharmacological and biophysical properties of this current show that it is not sustained by the K+ channels previously shown to be blocked by comparable concentrations of this compound, namely the Kv1.3 and Kv1.4; and also they show that the CP-sensitive I_{DRK} is sustained neither by other members of the Kv1 family nor by the Kv2 channels and Kv7 channels, whose affinity for CP had not been assessed; 3) within the MF subgroup of TG neurons, the CP-sensitive I_{DRK} expression strongly associates with nociceptive markers, such as capsaicin-sensitivity, small soma diameter, and long AHP; and 4) the CP-sensitive I_{DRK} prolongs the neuron AHP, and as result it strongly limits the neuron excitability, namely the firing frequency by holding for longer times the membrane potential at more negative values.

The voltage-gated K currents found in TG and DRG neurons have been classically divided into three distinct components, differently expressed in different neuronal subpopulations: slow inactivating transient K+ current (I_A), fast inactivating transient K+ current (I_K), and dominant sustained K+ current (I_D) (Everill et al. 1998; Everill and Kocsis 1999; Gold et al. 1996a; Matsumoto et al. 2010). The I_A has a fast (milliseconds) inactivation, insensitivity to TEA, and relatively low sensitivity to 4-AP (≥2 mM). The I_D differs from I_K for its enhanced sensitivity to lower concentrations of 4-AP (50–500 μM) and dendrotoxin (DTX; 0.001–1 μM), a blocker of some members of the Kv1 subfamily, and for the inactivation rate much slower than I_K. Finally, I_K is a sustained K current sensitive to relatively low (millimolar) concentrations of TEA and relatively insensitive to 4-AP. In adult rat DRG neurons ranging from 39 to 49 μm in diameter, Everill et al. (1998) identified three different combinations of K+ currents (I_A, I_K, and I_D currents, I_A and I_K currents, and I_K and I_D currents). By contrast, all adult rat TG neurons <27 μm in diameter possess all three types of current (Yoshida and Matsumoto 2005). In our previous work in adult mouse TG neurons we observed that, in terms of the three currents mentioned above, three distinct neuronal subpopulations could be clearly distinguished: large diameter (electrical capacitance >25 pF) neurons characterized by the coexpression of I_K and I_D, small diameter (capacitance <25 pF) neurons coexpressing I_K and I_A, and small diameter neurons expressing only I_K (Catacuzzeno et al. 2008). Several pharmacological results presented in this work indicate that the voltage-gated K current expressed in the latter subpopulation shares most of the properties of the I_K found in other sensory neurons. Namely, we found that this current was totally insensitive to the I_D current blocker DTX and to millimolar concentrations of 4-AP, while it could be sensibly blocked by TEA concentrations as low as 1 mM.

CP Blocks an I_{DRK} in a High Fraction of MF Sensory Neurons

CP is a dihydroquinoline compound developed by chemical modification of its related compound WIN 17317-3 in the attempt to find novel nonpeptide immunosuppressive agents that selectively blocked the Kv1.3 channels (Michne et al. 1995; Nguyen et al. 1996), highly and selectively expressed in T lymphocytes (Gutman et al. 2005), where they participate to the immunological response (Lewis and Cahalan 1995). Later studies showed however, that CP, as well as several related compounds, also blocked other channels such as Kv1.4 (Nguyen et al. 1996), widely expressed in neuronal and cardiac cells, where they participate in establishing the excitability properties (Cotzee et al. 1999), and, at higher concentrations, the HCN channels, underlying the pacemaker current of cardiac and neuronal cells (Lee et al. 2008). The data presented here indicate that in a subpopulation of mouse MF neurons CP blocks, at nanomolar concentration, an I_{DRK} not sustained by Kv1.3 or Kv1.4 channels. The conclusion that the Kv1.3 channel is not the molecular correlate of the CP-sensitive current originates from the insensitivity to MgTX, known to inhibit with high affinity these channels (Garcia-Calvo et al. 1993; Vicente et al. 2006). In addition, these channels do not appear to be expressed in sensory neurons (Rasband and Trimmer 2006). Kv1.4 channels could instead be excluded since 1) they display a fast inactivation not observed in the CP-sensitive current expressed in MF neurons; 2) DTX-I, an inhibitor of the Kv1.6 subunit that gives rise to nonactivating currents when associated to Kv1.4 subunit (Al-Sabi et al. 2011), was likewise ineffective; and 3) unlike the CP-sensitive current that is blocked by millimolar TEA concentrations and is only inhibited by 20% by 10 mM 4-AP, Kv1.4 channels have been found to be blocked by relatively low concentrations of 4-AP (IC_{50} = 13 μM) and to be insensitive to TEA (IC_{50} = 100 mM) (Gutman et al. 2005). The pharmacological data presented in this article, and in particular the insensitivity of the CP-sensitive current to XE991, also allow exclusion of the Kv7 channel family. This result is in accordance with the observation that the kinetics of Kv7 activation and deactivation are much too slow to be the CP-sensitive current. Kv7 current is also noninactivating so that it would very unlikely embody the slowly deactivating I_{DRK} current.

The biophysical properties found for the CP-sensitive current expressed in MF TG neurons strongly suggest that this current could be sustained by channels belonging to the Kv2 subfamily. The Kv2 subfamily of voltage-gated K channel α-subunits, composed of only two members (Kv2.1 and Kv2.2), is widely expressed in several types of neurons of the central nervous system, where it contributes to a major fraction of the delayed-rectifier K current (Du et al. 2000; Murakoski and Trimmer 1999). A distinctive property of Kv2 channels is represented by their relatively slow activation and deactivation kinetics, which make these channels particularly suited for controlling the neuronal MF response, rather than the repolarization of a single AP (Du et al. 2000). Kv2.1 channels can form heteromers with a number of modulatory α-subunits, such as Kv5.1, 6.1, 8.1, and 9.1–9.3, known as silent subunits because they do not form functional homomeric channels. Currents through heteromeric Kv2/silent subunits channels are biophysically very different from Kv2 homotetrameric channels, generally showing a hyperpolarizing shift in the activation and inactivation V_{1/2} and a slowing of the deactivation kinetics (Post et al. 1996; Kramer et al. 1998; Kerschensteiner and Stocker 1999). Some studies suggest the expression of Kv2.1 and Kv2.2 channel proteins and mRNA in rat DRG neurons and a downregulation of their expression following neuronal injury have been suggested to contribute to the injury-induced sensory neuron hyperexcitability (Kim et al. 2014).
A recent study reports the functional expression of Kv2 channels in mouse DRG neurons (Bocksteins et al. 2009). All these findings led us to suggest that the two components of the CP-sensitive current could be sustained by homomeric Kv2 channels or Kv2 heteromerized with some silent subunit. Unfortunately, when expressed in Xenopus oocytes, Kv2 transcripts generated an $I_{\text{DRK}}$ that was inhibited by CP with an affinity about one order of magnitude lower than in MF CP-sensitive neurons (cf. Fig. 2).

The expression of a CP-sensitive $I_{\text{DRK}}$ not sustained by Kv1.3 or Kv1.4 has also been reported in mouse pancreatic $\beta$-cells (Jacobson et al. 2010). The current, fully blocked by 5 $\mu$M CP, was in addition totally insensitive to toxins selective for Kv1.1-Kv1.3, Kv1.6, Kv3.2, Kv3.4, and Kv11.1 channels. These and our data strongly suggest the presence, at least in mice, of additional voltage-gated K$^+$ channels, other than Kv1.3 and Kv1.4, sensitive to low concentrations of CP. Although we are still unable to indicate the molecular nature of the CP-sensitive $I_{\text{DRK}}$ expressed in MF neurons, the data we obtained would exclude the Kv1.1-Kv4, Kv7, and Kv11 subfamilies, although the possibility remains that some silent modifier subunits may alter the sensitivity of these channels to CP.

**CP-Sensitive $I_{\text{DRK}}$ Segregates with MF Putative Nociceptors**

Our data show a marked segregation of the CP-sensitive $I_{\text{DRK}}$ in a subpopulation of mouse MF neurons displaying several nociceptive properties. More specifically, we found that a higher fraction of CP-sensitive than CP-insensitive neurons responded to bath application of capsaicin, the selective agonist of the vanilloid receptor TRPV1, selectively found in a subpopulation of polymodal nociceptors (Caterina 2007). We also found that CP-sensitive MF neurons displayed an electrical membrane capacitance significantly lower than CP-insensitive MF neurons, and thus they likely belong to the small body size C-type neurons, most of which are nociceptors (Lawson 2002). Finally, CP-sensitive neurons were found to have a longer AHP duration compared with CP-insensitive neurons, a property typically found in nociceptors. Lawson (2002) noticed that the electrophysiological properties associated with the nociceptive nature of sensory neurons fall into two classes. For class A properties, including long APs and slow firing rate, the difference between nociceptors and nonnociceptors is more pronounced with the slow deactivation velocity, so that a clear distinction can only be found within the C-type sensory class. The less common class B properties of nociceptive neurons, which include as best representative the AHP (Fang et al. 2005), do not correlate with conduction velocity of the axon, having similar values in C-, A$c$-, and A$a$/$\beta$-fiber nociceptive neurons. These properties are thus far more telling with regard to nociception than those of class A. Strict association between a long duration AHP and nociception had been originally reported on cultured DRG neurons from the adult rat by the group of Levine (Gold et al. 1996b), who showed that slow AHP expression was restricted to a subpopulation of putative nociceptors.

Our data do not allow to say whether the CP-sensitive current associates with nociceptors all over the TG neurons, as the study has been performed on a specific subpopulation of TG neurons (the MF subtype), and nothing can be said regarding the other neuronal subpopulations (i.e., DMF and SS neurons). The only conclusion that can be drawn from our data is that, within the MF neuronal subpopulation, that likely contains both nociceptive and nonnociceptive neurons, the CP-sensitive current correlates with the presence of several nociceptive markers.

**CP-Sensitive $I_{\text{DRK}}$ Underlies the Long AHP and as Result It Limits the Neuron Excitability**

Unlike the ionic conductances underlying the higher overshoot and longer AP duration (identified in the selective expression of the Nav1.8, voltage-gated Na channel; Blair and Bean 2002; Djouhri et al. 2003), the ionic basis of the long duration AHP in nociceptors has so far remained unclear. It needs to be emphasized that the AHP observed in sensory neurons belongs to the “fast” type (fAHP), as it follows as a direct continuation of the AP repolarization, and differs from the “medium” and “slow”-type AHP whose onset is usually preceded by a delay following the spike repolarization (Storm 1987). fAHP is generally observed in most central and peripheral neurons, where it has a duration in the order of tens of millisecond and may thus well contribute to neuron’s ability to discharge multiple APs during a prolonged depolarization or repetitive stimulation (Closer and Sather 2003). Since nociceptors are characterized by a fast AHP having a duration longer than that observed in nonnociceptive fibers, it is not surprising that this neuronal subpopulation has also a reduced fiber following frequency, that is the ability to respond with APs to high frequency stimulation (Lawson 2002). Interestingly, hindlimb inflammation has been reported to increase the MF ability of nociceptors by significantly reducing the maximal fiber following frequency (Djouhri et al. 2001), suggesting that a long duration AHP is critical in pain physiology. The fast kinetics provide strong clues regarding the underlying currents, which could not possibly be sustained by slowly activating or deactivated calcium-gated K currents found to strongly contribute to the slow AHP in many other tissues. In accordance, the specific inhibitors of the calcium-gated K channels IBTX and apamin were without effects under our recording conditions.

Notably, our pharmacological, electrophysiological, and modeling results strongly suggest that the slow deactivation that characterizes the $I_{\text{DRK}}$ component of the CP-sensitive $I_{\text{DRK}}$ is most likely responsible for the relatively long duration of the AHP preferentially found in CP-sensitive neurons. Thus the selective expression of the CP-sensitive $I_{\text{DRK}}$ in nociceptive neurons may be at least one of the reasons that determines the differences existing in the duration of the AHP between nociceptive and nonnociceptive neurons.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the author(s).
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AUTHOR CONTRIBUTIONS

Acot, M. C., Leonard RJ, Novick J, Stevens SP, Schmalhofer W, Garcia-Calvo M, Kaczorowski GJ, Garcia ML.


