Effects of $\alpha$-Dendrotoxin on $K^+$ Currents and Action Potentials in Tetrodotoxin-Resistant Adult Rat Trigeminal Ganglion Neurons

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ABSTRACT

To determine whether the $\alpha$-dendrotoxin ($\alpha$-DTX)-sensitive current ($I_D$) current, slow inactivating transient current ($I_A$), contributes to the modification of neuronal function in small-diameter adult rat trigeminal ganglion (TG) neurons insensitive to 1 $\mu$M tetrodotoxin (TTX), we performed two different types of experiments. In the voltage-clamp mode, two distinct $K^+$ current components, a fast inactivating transient current ($I_A$) and a dominant sustained current ($I_D$), were identified. $\alpha$-DTX (0.1 $\mu$M), ranging from 0.001 to 1 $\mu$M, maximally decreased $I_A$ by approximately 20% and $I_D$ by approximately 16.1% at a +50-mV step pulse, and 0.1 $\mu$M $\alpha$-DTX application increased the number of action potentials without changing the resting membrane potential. Irrespective of the absence and presence of 0.1 $\mu$M $\alpha$-DTX, applications of 4-aminopyridine (4-AP; 0.5 mM) and tetraethylammonium (TEA; 2 mM) inhibited approximately 50% inhibition of $I_A$ and $I_D$, respectively. 4-AP (0.5 mM) depolarized the resting membrane potential and increased the number of action potentials in the absence or presence of 0.1 $\mu$M $\alpha$-DTX. TEA prolonged the duration of action potentials in the absence or presence of 0.1 $\mu$M $\alpha$-DTX. These results suggest that $I_D$ contributes to the modification of neuronal function in adult rat TTX-resistant TG neurons, but after the loss of $I_D$ due to 0.1 $\mu$M $\alpha$-DTX application, 4-AP (0.5 mM) and TEA (2 mM) still regulate the intrinsic firing properties of action potential number and shape.

The D current, slow inactivating transient current ($I_D$) was first reported by Storm (1987) in hippocampal CA1 pyramidal neurons. The $I_D$ has been identified by its ability to delay the firing action potentials after a depolarizing current step and is sensitive to lower concentrations of $\alpha$-dendrotoxin ($\alpha$-DTX) and 4-aminopyridine (4-AP) (Storm, 1987; Coetzee et al., 1999). Furthermore, there is a report demonstrating that a local modulation of $I_D$ exists in the form of an endogenous mast cell degranulating peptide-like molecule in the dorsal root ganglion (DRG) neurons (Stansfeld and Feltz, 1988).

The trigeminal ganglion (TG) and DRG neurons express three distinct type of $K^+$ currents in varying quantities: dominant sustained $K$ current ($I_K$), fast inactivating transient $A$ current ($I_A$), and $I_D$ (Puil et al., 1989; Gold et al., 1996; Everill et al., 1998; Everill and Kocsis, 1999; Seifert et al., 1999). In adult rat DRG neurons ranging 39 to 49 $\mu$m in diameter, Everill et al. (1998) identified three different combinations of $K^+$ currents ($A$, $K$, and $D$ currents, $A$ and $K$ currents, and $K$ and $D$ currents) in the population of cells examined. Such a classification resembles a rapidly inactivating current, a slowly inactivating current, and a noninactivating current ($I_K$), as reported by McFarlane and Cooper (1991) in neonatal rat sensory neurons. Recent evidence has demonstrated that the small-diameter TTX-resistant (TTX-R) neurons isolated from the neonatal TG expressed $I_K$, $I_A$, and $I_D$ and that activation of GAB$A_B$ receptors inhibited the excitability due to the potentiation of $I_K$ and $I_A$ but not $I_D$ (Takeda et al., 2004). In comparison with properties of $K^+$ currents in TG neurons of embryonic and juvenile rats, Seifert et al. (1999) found a higher 4-AP sensitivity of sustained $K^+$ currents in the TG neuron of embryos, whereas the sensitivity of $I_A$ increased during development in juvenile animals. They also found that $I_D$ remained rather constant in TG neurons from a different age. However, there are no reports examining how $I_D$ functions in the adult rat TTX-R TG.

The purposes of the present study were designed to examine relative contribution of $I_D$ to other $K^+$ currents ($I_K$ and $I_A$)
and to assess their contribution to the firing properties of small-diameter adult rat TG neurons insensitive to TTX.

Materials and Methods

Cell Culture. For acute dissociation of the TG, adult Wistar rats (250–300 g) were deeply anesthetized with pentobarbital sodium (60 mg/kg i.p.) and were decapitated. A pair of the trigeminal ganglion were dissected and incubated in Hank's balanced salt solution (Invitrogen, Carlsbad, CA). They were incubated for 20 to 30 min at 35°C in Hank's balanced salt solution containing collagenase types XI (1 mg/ml; Sigma-Aldrich, St. Louis, MO) and I (1 mg/ml; Sigma-Aldrich). The cells were dissociated by triturating with a fire-polished Pasteur pipette and subsequently were plated onto poly-l-lysine-pretreated 35-mm dishes. The plating medium containing Leibovitz’s L-15 solution (Invitrogen) supplemented with 10% newborn calf serum (0.09 v/v), penicillin-streptomycin (50 U/ml) (Invitrogen), 26 mM NaHCO3, and 30 mM glucose. The cells were maintained in a humidified atmosphere of 95% air and 5% CO2 at 37°C. The cells were used for recording between 2 and 10 h after plating. Neurons were accepted for study only if they showed a stable resting membrane potential 40 mV, an action potential overshoot 20 mV, and a whole-cell capacitance 30 pF throughout the experiments.

Electrophysiology. Whole-cell patch-clamp recordings were performed at room temperature (22–25°C). The current was measured with an amplifier (Axopatch-1D; Axon Instruments Inc., Union City, CA). The pipette resistance was 2 to 5 MΩ with an amplifier (Axopatch-1D; Axon Instruments Inc., Union City, CA). The pipette resistances were 2 to 5 MΩ after filling with the recording solution. Currents were low-pass filtered at 5000 to 10,000 Hz with a 4-pole Bessel filter and digitally sampled at 25,000 to 100,000 Hz. After seal formation and membrane disruption, capacity transients (12–28 pF) were canceled, and a series resistance compensation (>80%) was employed. Isolated cells in the glass coverslip dish were placed in a recording chamber and visualized under the phase contrast on an inverted microscope (Nikon, Tokyo, Japan). Several drugs dissolved into the external solution were administered via a linear array of seven superfusion polyethylene tubes (280 μm in diameter) positioned closer to the cell bodies (approximately 200 μm).

V-Clamp Recording. After confirmation of TG neurons with the insensitivity to 1 μM TTX, we distinguished a transient K+ current (I_t) and a sustained K+ current (I_s) from the total K+ currents by using the same steps as described in a previous study (Everill and Kocsis, 1999). Outward K+ currents were elicited by stepping a conditioning pulse of either −40 or −120 mV from a holding potential of −80 mV; then, the membrane was depolarized from −40 or −120 mV to +50 mV in increments of 10 mV; +50 mV produced the largest peak in each recording. The I_t was determined by subtracting the −40-mV protocol from the −120-mV protocol. Activation of the currents was rapid and decayed only partially during 300-ms depolarization pulses. The slow inactivating K+ currents (I_{Ks}) were measured as the inhibited currents by application of α-DTX to I_s and I_A. The amplitude and rate of rise in the absolute current increased with increasing depolarization. In some experiments, 4-AP, tetrethylammonium chloride (TEA), and α-DTX were used to determine whether K+ currents in TTX-R TG neurons were antagonized by these blockers. We further determined which extent the K+ currents in TTX-R TG neurons are composed of I_A, I_K, and I_{Ks} and whether the I_{Ks} current is a subcomponent of the I_s.

1-Clamp Recording. For electrical stimulation, current injections were applied in increments of 50 pA. Action potentials in 34 TG neurons were initially evoked by a depolarizing pulse (45.9 ± 6.4 pA, 10–90 pA) for 300 ms and determined as 1 threshold. The number of action potentials was measured during 1 to 3 times threshold (1–3T) in the presence of TTX (1 μM). We classified them into two different categories, rapidly and slowly adapting TTX-R neurons, as described in a previous study (Mo and Davis, 1997). During current injection at 1–3T, the former fired one or two action potentials, but the latter fired multiple action potentials.

In some experiments, changes in action potential characteristics (resting membrane potential, firing frequency, duration of depolarization phase of action potential, and duration of half-amplitude of action potential) in response to current injections at 1–3T were examined before and after application of several K+ channel blockers (4-AP, TEA, and α-DTX) and their combination.

Recording Solution and Drugs. The normal external solution for the I-clamp experiments on action potentials contained 160 mM NaCl, 5 mM KCl, 10 mM HEPES, 1 mM MgCl2, 2 mM CaCl2, and 10 mM glucose, pH adjusted to 7.4 with NaOH. The normal internal solution contained 140 mM KCl, 10 mM HEPES, 10 mM EGTA, 1 mM CaCl2, 2 mM Mg-ATP, and 14 mM Na2 creatine phosphate, pH adjusted to 7.3 with KOH. For the V-clamp experiments on voltage-dependent K+ currents, the external solution contained 180 mM N-methyl-D-glucamine (NMDG), 5 mM KCl, 10 mM HEPES, 1 mM MgCl2, 2 mM CaCl2, and 10 mM glucose, pH adjusted to 7.4 with KOH, and the internal solution was the same as used for the I-clamp experiments. The estimates of free Ca2+ concentration ranged from 10 to 100 nM in the internal solution that added both EGTA (10 mM) and CaCl2 (1 mM). That concentration was normal Ca2+ in sensory neurons. Under such concentrations of EGTA and CaCl2, voltage-gated Ca2+ currents were successfully recorded in small TG neurons from neonatal rats (Ikeda and Matsumoto, 2003).

All drugs (stock solutions) were stored at −20°C and dissolved in standard and external NMDG-containing solutions. 4-AP, a selective I_A blocker (0.05–50 mM; Sigma-Aldrich), TEA, a selective I_K blocker (0.02–20 mM; Sigma-Aldrich), α-DTX, and selective I_{Ks} blocker (0.01–1 μM; Alomone Labs, Jerusalem, Israel) were added to the perfusion for a period ranging from 30 to 60 s.

All drugs and chemicals were dissolved in both external solutions prior to addition to bath solution. In experiments with higher concentrations of 4-AP and TEA (more than 1 mM), an equivalent amount of NMDG was removed from the external solutions.

Data Analysis. Data were collected and analyzed with Clampfit version 8.0 (Axon Instruments Inc.). Data were expressed as mean ± S.E. The group comparison of mean values was performed by the use of Student’s t-test and/or one-way analysis of variance with Tukey’s post hoc test for paired samples. P < 0.05 was considered statistically significant.

Results

TTX-R Neurons. Potassium currents were recorded from relatively small (<27 μm in diameter) TG neurons. The spike properties of these neurons were not significantly altered by 1 μM TTX application. This type of neuron was defined as a TTX-R TG neuron. The mean values for the cell diameters of recorded TTX-R neurons (n = 99) were 22.9 ± 0.2 μm, and the resting membrane potential was −61.2 ± 1.4 mV. The cell capacitance was 7.3 ± 0.9 pF.

Effects of α-DTX for I_A and I_K on the TTX-R TG Neurons. Before recordings of K+ currents, we initially confirmed that the recorded TG neuron was insensitive to 1 μM TTX. Separation of K+ currents was achieved by the response to variation in conditioning, and two distinct components of voltage-gated K+ currents, a sustained K+ current (I_s) and a transient K+ current (I_t), were identified (Fig. 1A). The neurons were first held at −80 mV, and they stepped to either −120 (Fig. 1A, top panel) or −40 (Fig. 1A, middle panel) mV for 300 ms (conditioning prepulse potential). Isolated outward currents were elicited by stepping from the conditioning prepulse potential to +50-mV increments of 10 mV. The I_A current was obtained by subtracting I_t from the total K+ current (Fig. 1A, bottom panel). In seven cells, the
peak $I_A$ was $6.5 \pm 1.6 \text{ nA}$ at the step pulse of $+50 \text{ mV}$, and the $I_K$ was $5.2 \pm 0.8 \text{ nA}$ at a $+50\text{ mV}$ step pulse. Those $K^+$ current recordings before and after the application of different concentrations of $\alpha$-DTX are shown in Fig. 1A.

$I_A$ and $I_K$ were relatively sensitive to $\alpha$-DTX. Figure 1, B and C, show the normalized current-voltage (I-V) relationship of $I_A$ and $I_K$, respectively. Figure 1D (bottom panel) shows inhibitory changes in $I_A$ and $I_K$ induced by $\alpha$-DTX applications at different concentrations ($0.001–1 \mu M$). $\alpha$-DTX applications concentration-dependently inhibited both $I_A$ and $I_K$. At a $50\text{ mV}$ step pulse, $0.001, 0.01, 0.1, \text{ and } 1 \mu M$ $\alpha$-DTX inhibited $5.3 \pm 2.7, 12.3 \pm 4.3, 21.8 \pm 4.1, \text{ and } 18.6 \pm 1.7\%$ of the baseline $I_A$ as well as $5.7 \pm 2.1, 12.6 \pm 1.3, 16.1 \pm 1.1, \text{ and } 14.1 \pm 1.1\%$ of the baseline $I_K$, respectively ($n = 7$). The application of $\alpha$-DTX at $0.1 \mu M$ caused a maximal inhibition of $I_A$, which was significantly larger than that of $I_K$ after the same concentration of $\alpha$-DTX.

**Effect of $\alpha$-DTX Application at Action Potentials in Small TTX-R TG Neurons.** We used $0.1 \mu M$ $\alpha$-DTX in this experiment because application of $0.1 \mu M$ $\alpha$-DTX was a maximal effect on the $K^+$ current modulation (Fig. 1). In I-clamp experiments, two different adaptation types of action potentials were observed (Fig. 2, A and C). In 22 cells, they belonged to the category of a slowly adapting type. As shown in Fig. 2A, cells of the slowly adapting type increased their frequency as the intensity of the depolarizing step pulses was increased, and $\alpha$-DTX application at $0.1 \mu M$ significantly enhanced the response of TG neurons to stepping pulses at $1–3T$ (Fig. 2B, a). Application of $0.1 \mu M$ $\alpha$-DTX had no or little effect on the resting membrane potential and the duration of half-amplitude of action potentials during intracellular injection of the currents ($1–3T$) (Fig. 2B, b and c), but the duration of depolarization phase of action potentials (DDP) was significantly reduced by $0.1 \mu M$ $\alpha$-DTX application (Fig. 2B, d). Figure 2C shows a typical example of the rapidly adapting type neuron that had no significant effect on the number of spikes during intracellular injection of the currents ($1–3T$), but that in this type of neurons ($n = 12$), the number of spikes increased after application of $0.1 \mu M$ $\alpha$-DTX (Fig. 2D, a). The resting membrane potential and the
duration of half-amplitude of action potentials during intracellular injection of 1–3T currents were not significantly altered by 0.1 μM α-DTX application (Fig. 2D, b and c), which did not cause any significant difference on the magnitude of decreased DDP provoked by stepping pulses at 1–3T (Fig. 2D, d).

Effects of 4-AP and α-DTX on I_A and Action Potential. Figure 3A, a, illustrates the effect of 4-AP (500 μM) on I_A. Five hundred micromolar 4-AP in seven cells inhibited 51.5 ± 3.0% of I_A at a +50-mV step pulse (Fig. 3A, b and c). Figure 3B, a, shows a typical example of the effects of application of 0.1 μM α-DTX in the absence and presence of 4-AP (500 μM) on I_A in a different cell group. The I_A in a TTX-R TG neuron was activated at a potential between −70 and −60 mV and increased with depolarization. After 0.1 μM α-DTX application, the I_A was reduced at the potentials more depolarized than −60 mV. The I_A was further reduced after coapplication of 0.1 μM α-DTX and 0.5 mM 4-AP, and the activation of the current occurred at the same potential seen after α-DTX application only (Fig. 3B, a and b). The summarized results in seven cells are shown in Fig. 3B, c. The I_A was significantly inhibited by application of 0.1 μM α-DTX (20.0 ± 4.4%), and the subsequent additional application of 0.5 mM 4-AP to the α-DTX perfusion resulted in a further inhibition of I_A (53.3 ± 3.6%). The magnitude of inhibited I_A after application of both α-DTX (0.1 μM) and 4-AP (0.5 mM) was similar to that after 4-AP application (0.5 mM) only in the different cell group.

To determine whether I_D is a subcomponent of I_A on the responses of TTX-R TG neuronal activity, showing slowly
and rapidly adapting types, to 3T current injection, we examined changes in the properties of their activity in response to α-DTX (0.1 μM) application in the absence and presence of 4-AP (0.5 mM). During the depolarizing step pulse (180 pA, 300 ms), as shown in Fig. 4A, TTX-R TG neurons fired repeatedly, and this type of firing behavior belonged to the category of slowly adapting neurons. The application of 0.1 μM α-DTX did not significantly change the membrane potential (RMP) but increased the number of action potentials. The subsequent additional application of 0.5 mM 4-AP slightly increased the RMP but caused a further increase in the neuronal activity. The summarized results in seven TTX-R TG neurons are shown in Fig. 4B, a to c. The application of 0.1 μM α-DTX at a maximal concentration to inhibit $I_K$ could increase action potentials, resulting in the reduction in the DDP. Such an effect became more prominent by additional application of 0.5 mM 4-AP, and the responses were usually associated with an increase in the RMP (Fig. 4C). α-DTX application (0.1 μM) also increased the number of action potentials in a rapidly adapting type neuron, and this potential was not accompanied by any significant change in the DDP. Under these conditions, subsequent addition of 4-AP (0.5 mM) significantly increased the RMP and caused a further increase in action potentials, which was characterized by a decrease in the DDP (Fig. 4C, a–c).

**Effects of α-DTX and TEA on $I_K$ and Action Potential.** To further characterize $I_D$ of TG neurons, we used TEA. As shown in Fig. 5A, a to c, 2 mM TEA application inhibited the amplitudes of $I_K$ by approximately 51.5 ± 3.0% ($n = 7$). The $I_K$ was significantly reduced after 0.1 μM α-DTX application (16.3 ± 7.1%) and further reduced after coapplication of 0.1 μM α-DTX and 2 mM TEA (49.8 ± 8.9%) (Fig. 5B, a–c). The magnitude of inhibited $I_K$ after coapplication of α-DTX (0.1 μM) and TEA (2 mM) was similar to that after TEA application (2 mM) only in the different cell group. When considering Figs. 3 and 5, taken together, it is possible to speculate that there were no significant differences of α-DTX (0.1 μM) effects on $I_A$ and $I_D$.

To determine whether $I_D$ is a subcomponent of $I_K$ on the response of TTX-R TG neuronal activity, showing slowly and rapidly adapting type, to a 3T current injection, we examined changes in the properties of their activity in response to α-DTX (0.1 μM) application in the absence and presence of TEA (2 mM). During the depolarizing step pulse (210 pA, 300 ms), as shown in Fig. 6A, the TTX-R TG neurons fired repeatedly, and this neuron was a slowly adapting type. α-DTX application (0.1 μM) that did not significantly alter the RMP increased the firing rates. The subsequent additional application of 2 mM TEA also increased the number of action potentials, and this increase was due to the reduction in the DDP. Additional application of 2 mM TEA still increased the number of action potentials, but this increased
activity was lower than that seen after α-DTX application only, and the response was usually associated with a longer duration of half-amplitude. α-DTX application (0.1 μM) also increased the number of action potentials in a rapidly adapting type neuron, subsequent application of TEA (2 mM) significantly reduced the firing rates due to a longer duration of the action potential (Fig. 6). Although the number of action potentials was increased after 0.1 μM α-DTX application only, additional application of 4-AP and TEA could modulate α-DTX-induced increase in the TTX-R TG neuronal activity, resulting in the alteration of their firing properties induced by two K⁺ channel blockers (Figs. 4 and 6).

Discussion

The cell bodies located in the TG are thought to relay the sensory information from the peripheral to the central nervous system. Despite the absence of synaptic inputs in the TG neurons, their perikarya modulate afferent signal transduction (Puil et al., 1989). In sensory neurons, the TTX-R sodium current that is not significantly affected by micromolar concentrations of TTX is expressed in C-fiber-type neurons (Yoshida et al., 1978; Strassman and Raymond, 1999).

There is a positive correlation between the neuronal cell size and axonal conduction velocity of Aδ- or C-fiber afferents.
in rat DRG neurons (Harper and Lawson, 1985). TG neurons recorded in this study were below 25 μm in diameter and also insensitive to 1 μM TTX application. Furthermore, the action potentials at graded depolarizing step pulses revealed either repetitive firings, which were distinguished by a difference in the spike frequency and in the duration of depolarizing phase of action potential, or single action potentials. These spikes had a marked inflection or hump in the repolarization phase, as reported in TTX-R DRG or neonatal mice TG neurons (Yoshimura et al., 1996; Cabanes et al., 2002), indicating that TTX-R TG neurons that revealed repetitive firings may preserve information concerning the stimulus magnitude of small-diameter TTX-R TG neurons.

Two transient K⁺ currents have been reported in TG and DRG neurons (Everill et al., 1998; Everill and Kocsis, 1999; Takeda et al., 2004): fast inactivating current (Iₐ) and slow inactivating current (Iᵦ). Iᵦ is different from Iₐ, showing an enhanced sensitivity to lower concentrations of 4-AP (50–500 μM) and α-DTX (0.001–1 μM) in DRG and TG neurons (Wu and Barish, 1992; Everill et al., 1998; Everill and Kocsis, 1999; Takeda et al., 2004). α-DTX-sensitive K⁺ currents were expressed by Kv1.1, Kv1.2, and Kv1.6 in rat nodose ganglion neurons (Glazebrook et al., 2002). Selective blockade of Iᵦ by α-DTX was 1 to 2 μM in embryonic mouse hippocampal neurons (Wu and Barish, 1992) and 10 to 100 nM in nodose ganglion neurons (Glazebrook et al., 2002). In this study, the minimal concentration of α-DTX examined (0.001–1 μM) that evoked a maximal inhibition of Iᵦ was 0.1 μM, and this concentration of α-DTX resulted in a significant reduction in both Iₐ (approximately 22% of control) and Iᵦ (approximately 16% of control) at a +50-mV step pulse. Furthermore, TTX-R TG neurons in the loss of Iᵦ due to 0.1 μM α-DTX application had a much larger Iₐ component compared with the blockade of Iᵦ. Even in the presence of 0.1 μM α-DTX, other K⁺ currents are still present, and these currents would act to compensate the loss of the Iᵦ. In this study, we demonstrated that 0.5 mM 4-AP and 2 mM TEA application caused approximately 50% inhibition of the Iₐ and Iᵦ, respectively, irrespective of the absence or presence of 0.1 μM α-DTX, indicating that the remaining 4-AP- and TEA-sensitive K⁺ currents are still present in small-diameter TG neurons after blockade of Iᵦ. In other words, the Iᵦ is one class of transient K⁺ currents as well as sustained K⁺ currents. Indeed, there is evidence that the sensitivity of K⁺ currents to 4-AP and TEA in rat small-diameter DRG neurons, 2 mM 4-AP application caused an 80% reduction in Iₐ but had no significant effect on Iᵦ, whereas 10 mM TEA produced a 70% inhibition.

Fig. 5. Effect of TEA in the absence or presence of α-DTX on Iᵦ. A, typical effect of TEA (2 mM) application in the absence of α-DTX (0.1 μM) on Iᵦ (a) and summary of TEA (2 mM) on the normalized I-V relationship (b). c, percentage of inhibition of Iᵦ by TEA (2 mM). Values show mean ± S.E.M. *, P < 0.05 (n = 5) versus control. B, typical effect of TEA (2 mM) in the presence of α-DTX (0.1 μM) (a) and summary of TEA (2 mM) and α-DTX (0.1 μM) effect on the normalized I-V relationship (b). c, the percentage of inhibition of Iᵦ by α-DTX (0.1 μM) in the absence (□) and presence (■) of TEA (2 mM). Values show mean ± S.E.M., * P < 0.05 (n = 5) versus control; #, P < 0.05 versus after TEA application.
of $I_K$ but did not change $I_A$ significantly (Fedulova et al., 1998).

In current-clamp experiments, 0.1 μM α-DTX application had little or no effect on the resting membrane potential but increased the number of action potentials in both slowly and rapidly adapting type TTX-R TG neurons. The former effect was accompanied by a decrease in the duration of depolarizing phase of action potential, indicating that the threshold for the first action potential was reduced by α-DTX application. Interestingly, this enhanced activity of TTX-R TG neurons was similarly manifested by increasing the threshold currents required to evoke action potentials in the absence of α-DTX. When considering the responsiveness to rapidly adapting type TTX-R TG neurons to 0.1 μM α-DTX, $I_D$ contributed to the adaptation of neuronal responses to the depolarizing step. The results are in agreement with the observations demonstrating that $I_D$ has the ability to modulate the frequency and adaptation of action potentials in peripheral and central sensory neurons (Glazebrook et al., 2002; Mo et al., 2002). Blockade of $I_A$ with 4-AP (500 μM) in the presence of α-DTX (0.1 μM) caused a further increase in the action potential in both slowly and rapidly adapting type TTX-R TG neurons, and the responses were usually associated with elevation of the resting membrane potential and a decrease in the duration of depolarizing phase of action potentials. Under these conditions, the duration of half-amplitude of the first action potential was not significantly altered by the combination of 4-AP and α-DTX applications. The results

Fig. 6. Effects of α-DTX on responses of TTX-R TG neurons before and after TEA.
A, typical action potentials were induced by 3T current injection (210 pA) after 1 μM TTX (control, left), 0.1 μM α-DTX (middle), and 2 mM TEA + 0.1 μM α-DTX in the slowly adapting type TG neuron. Right panel, first action potentials after TTX (control), α-DTX, and TEA + α-DTX. B, changes in the number of spikes (a), resting membrane potential (b), duration of half-amplitude of action potential (c), and DDP (d) in response to α-DTX in absence and presence of TEA. C, action potentials were induced by a 3T current injection (180 pA) after 1 μM TTX (control, left), 0.1 μM α-DTX (middle), and 2 mM 4-AP + 0.1 μM α-DTX application in the rapidly adapting type TG neuron. Right panel, first action potentials after TTX (control), α-DTX, and TEA + α-DTX. D, changes in the number of spikes (a), resting membrane potential (b), duration of half-amplitude of action potential (c), and DDP (d) in response to α-DTX in the absence and presence of TEA. Values show mean ± S.E.M. *, $P < 0.05$ (n = 7) versus control; #, $P < 0.05$ (n = 7) versus after TEA application.
lead us to suggest that $I_A$ was a more effective current to increase the number of action potentials, particularly in the case with the loss of $I_D$. On the other hand, TEA (2 mM) application in the presence of $\alpha$-DTX (0.1 $\mu$M) decreased the discharge of action potentials but increased the duration of them. We also found that additional TEA application did not significantly alter the resting membrane potential in the presence of $\alpha$-DTX. These results suggest that characteristic changes in the action potential wave form and discharge rate in responses to 4-AP (500 $\mu$M) changes in the action potential wave form and discharge rate in the presence of $\alpha$-DTX predominantly reflect a selective blockade of $I_A$ and $I_K$, respectively, in small-diameter adult TTX-R TG neurons. The two-component outward currents in the large cutaneous afferent DRG neurons were reduced after nerve ligation ($I_A$ by 60% and $I_K$ by 65%, compared with control cell in the DRG), but $I_D$ was not significantly reduced after ligation (Everill and Kocsis, 1999). As suggested by Cabanes et al. (2003) in the guinea pig TG neurons, we cannot completely rule out the possibility that a further increase in the action potential frequency induced by the depolarizing step pulse may develop the sensitivity to temperature (approximately 25°C) of the external solution in TTX-R TG neurons after coapplication of 4-AP (500 $\mu$M) and $\alpha$-DTX (0.1 $\mu$M).

In conclusion, our results demonstrate that $I_D$ contributes to the modification of neuronal function in adult rat TTX-R TG neurons via inhibition of both $I_A$ and $I_K$ and that this modification was not associated with any significant change in the resting membrane potential. After the loss of $I_D$, 50% inhibition of $I_A$ (0.5 mM 4-AP) and $I_K$ (2 mM TEA) still regulates the intrinsic firing properties of the action potential number and timing.

References


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