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

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ABBREVIATIONS: α-DTX, alpha-dendrotoxin; TG, trigeminal ganglion; DRG, dorsal root ganglion; TTX, tetrodotoxin; TTX-R, tetrodotoxin-resistance; I_A, a fast inactivating transient current; I_K, a dominant sustained current; I_D, slow inactivating transient current; NMDG, N-methyl D-glucamine; 4-AP, 4-aminopyridine; TEA, tetraethylammonium; I-V, current-voltage; RPM, resting membrane potential; DDP, duration of depolarizing phase of action potentials;

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ABSTRACT

To determine whether the α-dendrotoxin (α-DTX) sensitive current (a slow inactivating transient current, D-current, I_D) contributes to the modification of neuronal function in small-diameter adult rat trigeminal ganglion (TG) neurons insensitive to 1μM 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_K), were identified. Alpha-DTX (0.1μM) ranging from 0.001 to 1μM, maximally decreased I_A by approximately 20%, and I_K by approximately 16.1% at a +50mV step pulse, and 0.1μM α-DTX application increased the number of action potentials without changing the resting membrane potential. Irrespective of the absence and presence of 0.1μM α-DTX, applications of 4-aminopyridine (4-AP, 0.5mM) and tetraethylammonium (TEA, 2mM) inhibited approximately 50% inhibition of I_A and I_K, respectively. 4-AP (0.5mM) depolarized the resting membrane potential and increased the number of action potentials in the absence or presence of 0.1μM α-DTX. TEA prolonged the duration of action potentials in the absence or presence of 0.1μM α-DTX. These results suggest that I_D contributes to the modification of neuronal function in adult rat TTX-R TG neurons, but after the loss of I_D due to 0.1μM α-DTX application, 4-AP (0.5mM) and TEA (2mM) still regulate the intrinsic firing properties of action potential number and shape.

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Introduction

The D-current (I_D) was first reported by Storm (1987) in hyppocampal 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 α–dentrotoxin (α-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 (MCD)-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 slow inactivating transient (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 39-49μm in diameter, Everill et al. (1998) identified three different combinations of K^+ currents (A-, K- and D-current, A- and K-current, K- and D-current) in the population of cells examined. Such a classification resembles a rapidly inactivating current (I_{Af}), a slowly inactivating current (I_{As}), 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 $GABA_B$ 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, while the sensitivity of I_A increased during development in juvenile animals. They also found that I_D remained rather constant in TG neurons from different age. But there are no reports examing 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 or small-diameter adult rat TG neurons insensitive to TTX.

Materials and Methods

Cell Culture. For acute dissociation of the trigeminal ganglion (TG), adult Wistar rats (250-300g) were deeply anesthetized with pentobarbital sodium (60mg/kg i.p.) and were decapitated. A pair of the trigeminal ganglion were dissected and incubated in Hank's balanced salt solution (HBSS) (Invitrogen Corp., Carlsbad, CA USA). They were

incubated for 20-30 min at 35 ºC in HBSS containing collagenase typeXI (1mg/ml SIGMA Chemical Co., St. Louis, MO) and typeI (1mg/ml Sigma Chemical Co.). The cells were dissociated by trituration with a fire-polished Pasteur pipette and subsequently were plated onto poly-L-lysine pretreated 35mm dishes. The plating medium contained Leibovitz's L-15 solution (Invitorogen Corp) supplemented with 10% newborn calf serum (0.09v/v), penicillin-streptomycin (50U/ml) (Invitrogen Corp), 26mM $NaHCO_3$ and 30mM glucose. The cells were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37C. The cells were used for recording between 2 and 10 hr after plating. Neurons were accepted for study only if they showed a stable resting membrane potential < -40mV, an action potential overshoot > +20mV, and a whole cell capacitance < 30pF, throughout the experiments.

Electrophysiology. Whole-cell patch-clamp recordings were performed at room temperature (22-25ºC). The current was measured with an amplifier (Axopatch-1D, Axon instruments, Forster City, CA). The pipette resistance was 2-5MΩ after filling with the recording solution. Currents were low-pass filtered at 5-10K Hz with a four pole Bessel filter and digitally sampled at 25-100K Hz. After seal formation and membrane disruption, capacity transients (12-28pF) were cancelled and a series resistance compensation (>80%) was employed. Isolated cells in the glass cover slip 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 (280μm in diameter) positioned closer to the cell bodies (approximately 200μm).

V-Clamp Recording. After confirmation of TG neurons with the insensitivity to 1μM TTX, we distinguished a transient K^+ current (I_A) and a sustained K^+ current (I_K) 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 –40mV or –120mV from a holding potential of –80mV; then the membrane was depolarized from –40mV or –120mV to +50mV in increments of 10mV; +50mV produced the largest peak in each recording. The I_A was determined by subtracting the –40mV protocol from the –120mV protocol. Activation of the currents was rapid and decayed only partially during 300ms depolarization pulses. The slow inactivating K^+ current (I_D) was measured as the inhibited currents by application of alpha-dendrotoxin (α-DTX) on I_K and I_A. The amplitude and rate of rise in the absolute current increased with increasing depolarization. In some experiments, 4-aminopyridine (4-AP), tetraethylammonium 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 to what extent the K^+ currents in TTX-R TG neurons are composed of I_A, I_K and I_D, and whether the I_D current is

a subcomponent of the I_A.

I-Clamp Recording. For electrical stimulation, current injections were applied in increments of 50pA. Action potentials in 34 TG neurons were initially evoked by a depolarizing pulse (45.9±6.4pA, 10-90pA) pulses for 300ms and determined as one threshold (1T). The number of action potentials was measured during 1-3 times threshold (1-3T) in the presence of TTX (1μM). 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 (in mM): 160 NaCl, 5 KCl, 10 HEPES, 1 $MgCl_2$, 2 $CaCl_2$, 10 glucose pH adjusted to 7.4 with NaOH. The normal internal solution contained (in mM): 140 KCl, 10 HEPES, 10 EGTA, 1 $CaCl_2$, 2 Mg-ATP, 14 Na_2 creatine

phosphate, adjusted to 7.3 with KOH. For the V-clamp experiments on voltage-dependent K^+ currents, the external solution contained (in mM): 180 N-methyl D-glucamine (NMDG), 5 KCl, 10 HEPES, 1 $MgCl_2$, 2 $CaCl_2$, 10-glucose pH adjusted to 7.4 with KOH, and the internal solution was the same as used for the I-clamp experiments. Although internal solutions included 10mM EGTA, to concentration of Ca^{2+} ranged 10-100nM in the internal solution that added 1mM $CaCl_2$. That concentration was normal Ca^{2+} in sensory neurons. Under such concentrations of EGTA and $CaCl_2$, voltage-gated Ca^{2+} currents were successfully recorded in small TG neurons from neonatal rats (Ikeda and Matsumoto, 2003).

All drugs (stock solutions) were stored at –20ºC and dissolved in standard and external NMDG-containing solutions. 4-AP, a selective I_A blocker (0.05-50mM, SIGMA Chemical Co.), TEA, a selective I_K blocker (0.02-20mM, Sigma.), α-DTX, selective I_D blockers (0.01-1μM, Alomone Labs Ltd. Jerusalem, Israel.) were added to the perfusion for a period ranging from 30 to 60s.

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 1mM), an equivalent amount of NMDG was removed from the external solutions.

Data Analysis. Data were collected and analyzed with the Clamp fit v8.0 (Axon

Instruments. Data were expressed as mean ± SE. The group comparison of mean values was performed by the use of Student's t test and/or one-way ANOVA with Tukey's post hoc test for paired samples. A P value less than 0.05 was considered statistically significant.

RESULTS

TTX-R Neurons. Potassium currents were recorded from relatively small (<27μM in diameter) TG neurons. The spike properties of these neurons were not significantly altered by 1μM 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±0.2μm, and the resting membrane potential was –61.2±1.4mV. The cell capacitance was 7.3±0.9pF.

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 1μM 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_K) and a transient K^+ current (I_A), were identified (Fig.1 A). The neurons were first held at –80mV and they stepped to either –120mV (Fig.1 A, upper panel) or –40mV (Fig. 1 A middle panel) for 300ms (conditioning prepulse potential). Isolated outward currents were elicited

by stepping from the conditioning prepulse potential to +50mV increments of 10mV. The I_A current was obtained by subtracting I_K from the total K^+ current (Fig.1 A, lower panel). In 7 cells, the peak I_A was 6.5±1.6nA at the step-pulse of +50mV, the I_K was 5.2±0.8nA at a +50mV step-pulse. Those K^+ current recordings before and after the application of different concentrations of α-DTX are shown in Fig. 1 A.

I_A and I_K were relatively sensitive to α-DTX. Figure.1 B and C show the normalized current-voltage (I-V) relationship of I_A and I_K, respectively. Figure 1 D (lower panel) shows inhibitory changes in I_A and I_K induced by α-DTX applications at different concentrations (0,001-1μM). Alfa-DTX applications concentration-dependently inhibited both I_A and I_K. At a 50mV step pulse, 0.001μM, 0.01μM, 0.1μM,and 1μM of α-DTX inhibited 5.3±2.7%, 12.3±4.3%, 21.8±4.1% and 18.6±1.7 of the baseline I_A as well as 5.7±2.1%, 12.6±1.3%, 16.1±1.1% and 14.1±1.1 of the baseline I_K, respectively (n=7). The application of α-DTX at 0.1μM caused a maximal inhibition of I_A, which was significantly larger than that of I_K after the same concentration of α-DTX.

Effect of α-DTX Application at Action Potentials in Small TTX-R TG Neurons. We used 0.1μM α-DTX in this experiment, because application of 0.1μM α-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, C). In 22 cells, they

belonged to the category of a slowly adapting type. As shown in Figure.2 A, cells of the slowly adapting type increased their frequency as the intensity of the depolarizing step pluses was increased, and α-DTX application at 0.1μM significantly enhanced the response of TG neurons to stepping pulses at 1-3threshold (T) (Fig. 2 Ba). Application of 0.1μM α-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.2 Bb, Bc), but the duration of depolarization phase of action potentials (DDP) was significantly reduced by 0.1μM α-DTX application (Fig.2 Bd). Fig.2 C 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 0.1μM α-DTX (Fig. 2Da). 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 0.1μM α-DTX application (Fig. 2Db, Dc), which did not cause any significant difference on the magnitude of decreased DDP provoked by stepping pulses at 1-3T (Fig. 2Dd).

Effects of 4-AP and α-DTX on I_A and Action Potential. Figure.3 Aa illustrates the effect of 4-AP (500μM) on I_A. Five hundred μM 4-AP in 7 cells inhibited 51.5±3.0% of I_A at a +50mV step-pulse (Fig.3 Ab, Ac). Figure.3 Ba shows a typical example of the effects

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of application of α-DTX (0.1μM) in the absence and presence of 4-AP (500μM) 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 –60mV and increased with depolarization. After 0.1μM α-DTX application the I_A was reduced at the potentials more depolarized than –60mV. The I_A was further reduced after co-application of 0.1μM α-DTX and 0.5mM 4-AP, and the activation of the current occurred at the same potential seen after α-DTX application only (Fig. 3Ba, Bb). The summarized results in 7 cells are shown in Fig. 3 Bc. The I_A was significantly inhibited by application of 0.1μM α-DTX (20.0±4.4%), and the subsequent additional application of 0.5mM 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 (0.1μM) and 4-AP (0.5mM) was similar to that after 4-AP application (0.5mM) 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 (0.1μM) application in the absence and presence of 4-AP (0.5mM). During the depolarizing step pulse (180pA 300ms), as shown in Fig. 4 A, TTX-R TG neurons fired repeatedly and this type of firing behavior belonged to the category of slowly adapting neurons. The application of 0.1μM α-DTX did not significantly change the resting membrane potential

(RMP) but increased the number of action potentials. The subsequent additional application of 0.5mM 4-AP slightly increased the RMP but caused a further increase in the neuronal activity. The summarized results in 7 TTX-R TG neurons are shown in Fig. 4 Ba-c. The application of 0.1μM α-DTX at a maximal concentration to inhibit I_A could increase action potentials, resulting in the reduction in the DDP. Such an effect became more prominent by additional application of 0.5mM 4-AP, and the responses were usually associated with an increase in the RMP (Fig. 4 C). Alpha-DTX application (0.1μM) 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 (0.5mM) significantly increased the RMP and caused a further increase in action potentials, which was characterized by an decrease in the DDP (Fig. 4 Ca-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. 5 Aa, Ab, Ac, 2mM TEA application inhibited the amplitudes of I_K by approximately 51.5±3.0% (n=7). The I_K was significantly reduced after 0.1μM α-DTX application （16.3±7.1%）and further reduced after co-application of 0.1μM α-DTX and 2mM TEA (49.8±8.9%) (Fig. 5 Ba, Bb, Bc). The magnitude of inhibited I_K after co-application of α-DTX (0.1μM) and TEA (2mM) was similar to that after TEA

application (2mM) only in the different cell group. When considering Fig.3 and 5, taken together, it is possible to speculate that there were no significant differences of α-DTX (0.1μM) 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 responses to α-DTX (0.1μM) application in the absence and presence of TEA (2mM). During the depolarizing step pulse (210pA, 300ms), as shown in Fig. 6 A, the TTX-R TG neurons fired repeatedly and this neuron was a slowly adapting type. Alpha-DTX application (0.1μM) that did not significantly alter the RMP level increased the firing rates. The subsequent additional application of 2mM TEA had no significant effect on RMP but decreased their firing rate. The response was associated with a longer duration of half-amplitude. The summarized results in 7 TTX-R TG neurons are shown in Fig. 6 Ba-d. Alpha-DTX application at 0.1μM increased the number of action potentials and this increase was due to the reduction in the DDP. Additional application of 2mM 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. Alpha-DTX application (0.1μM) also increased the number of action potentials in a rapidly adapting type neuron, subsequent application

of TEA (2mM) 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 0.1μM 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 (Fig4, 6).

Discussion

The cell bodies located in the trigeminal ganglion (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 modulates afferent signal transudation (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 (Happer and Lawson, 1985). TG neurons recorded in this study were below 25μm in diameter and also insensitive to 1μM 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 as well as 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., 2003); fast inactivating current (I_A) and slow inactivating current (I_D). I_D is different from I_A, showing an enhanced sensitivity to lower concentrations of 4-AP (50-500μM) and α-DTX (0.001-1μM) in DRG and TG neurons (Wu and Barish, 1992; Everill et al., 1998; Everill and Kocsis, 1999; Takeda et al., 2003; 2004). Alpha-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 I_D by α-DTX was 1-2μM in embryonic mouse hippocampal neurons (Wu and Barish, 1992), and 10-100nM in nodose ganglion neurons (Glazebrook et al., 2002). In this study, the minimal concentration of α-DTX examined (0.001-1μM), which evoked a maximal inhibition of I_D, was 0.1μM and this concentration of α-DTX resulted in a significant reduction in both I_A

(approximately 22% of control) and I_K (approximately 16% of control) at a +50mV step pulse. Furthermore, TTX-R TG neurons in the loss of I_D due to 0.1μM α-DTX application had a much larger I_A component compared with the blockade of I_K. Even in the presence of 0.1μM α-DTX, other K^+ currents are still present and these currents would act to compensate the loss of the I_D. In this study, we demonstrated that 0.5mM 4-AP and 2mM TEA application caused approximately 50% inhibition of the I_A and I_K, respectively, irrespective of the absence or presence of 0.1μM α-DTX, indicating that the remaining 4-AP- and TEA- sensitive K^+ currents are still present in small diameter TG neurons after blockade of I_D. In other word, the I_D 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, 2mM 4-AP application caused an 80% reduction in I_A but had no significant effect on I_K, whereas 10mM TEA produced a 70% inhibition of I_K but did not change I_A significantly (Fedulova et al., 1998).

In current-clamp experiments, 0.1μM 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 considered the responsiveness to rapidly adapting type TTX-R TG neurons to 0.1μM 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 (500μM) in the presence of α-DTX (0.1μM) 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 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 (2mM) application in the presence of α-DTX (0.1μM) deceased 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 α-DTX. These results suggest that characteristic changes in the

action potential waveform and discharge rate in responses to 4-AP (500μM) and TEA (2mM) applications in the presence of α-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 large cutaneous afferent DRG neurons were reduced after nerve ligation; I_A by 60%, 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 25ºC) of the external solution in TTX-R TG neurons after co-application of 4-AP (500μM) and α-DTX (0.1μM).

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 (4-AP, 0.5mM) and I_K (TEA, 2mM) still regulate the intrinsic firing properties of the action potential number and timing.

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Legends of Figures

Fig. 1. Effects of α-DTX on I_A and I_K. (A), Typical examples of three outward voltage-gated K^+ currents (total, I_K and I_A) after α-DTX applications (0.001, 0.01, 0.1 and 1μM). Right panels show pulse protocols. I_A was determined by subtraction of I_K from the total outward K^+ current. Summary of α-DTX (0.001- 1μM) effects on the normalized I-V relationships in I_A (B) and I_K (C). (D), The percent inhibition of I_A (□) or I_K (■) after application of α-DTX (0.001-1μM). Values show mean ± S.E.M. *, $P<0.05$ (n=6) versus control (I_A), **, $P<0.05$ (n=6) versus control (I_K), #, $P<0.05$ versus I_K.

Fig. 2. Effect of α-DTX application on the responses of slowly and rapidly adapting type TG neurons to current injections. (A), The action potentials was induced by a 3 threshold (T) current injection (180pA) after 1μM TTX application in the absence and presence of 0.1μM α-DTX in the slowly adapting type TG neuron (A). Right panel of (A) shows the first action potentials after TTX application in the absence and presence of α-DTX. (B), Changes in the number of spikes (a), resting membrane potential (b), duration of half amplitude of action potentials and duration of depolarizing phase of action potentials (DDP) in response to 1-3T current injections. (C), Typical action potentials were induced by a 3T current injection (150pA) after 1μM TTX application in the absence and presence of 0.1μM α-DTX in the rapidly adapting type TG neuron (C).

Right panel of (C) shows the first action potential potentials after TTX application in the absence and presence of α-DTX. (D), Changes in the number of spikes (a), resting membrane potential (b), duration of half amplitude of action potentials and DDP in response to 1-3T current injections. Values show mean ± S.E.M. *, P<0.05 (n=6) versus control, #, P<0.05 (n=6) versus α-DTX application.

Fig. 3. Effects of 4-AP in the absence or presence of α-DTX on I_A. (A), A typical effect of 4-AP (0.5mM) application on I_A (a) and summary of 4-AP (0.5mM) effect on the normalized I-V relationship (b). The percent inhibition of I_A by 4-AP (0.5mM) (c). Values shows mean ± S.E.M. *, P<0.05 (n=7) versus control. (B), A typical effect of 4-AP (0.5mM) application in the presence of α-DTX (0.1μM) (a) and summary of 4-AP (0.5mM) and α-DTX (0.1μM) effect on the normalized I-V relationship (b). The percent inhibition of I_A by α-DTX (0.1μM) in the absence (□) and presence (■) of 4-AP (0.5mM) (c). Vertical bars are show mean ± S.E.M. (n=7). Values show mean ± S.E.M. *, P<0.05 (n=7) versus control, #, P<0.05 (n=7) versus after 4-AP application.

Fig. 4. Effects of α-DTX on the responses of TTX-R TG neurons before and after 4-AP. (A), The action potentials were induced by a 3 threshold (T) current injection (180pA) after 1μM TTX (control, left), 0.1μM α-DTX (middle) and 0.5mM 4-AP+0.1μM α-DTX in the slowly adapting type TG neuron. Right panel shows the first action potentials after

TTX (control), α-DTX, and α-DTX+4-AP. (B), Changes in the number of spikes (a), resting membrane potential (b), duration of half amplitude of action potential (c) and duration of depolarizing phase of action potentials (DDP)(d) in response to α-DTX in the absence and presence of 4-AP. (C), The action potentials were induced by a 3T current injection (180pA) after 1μM TTX (control, left), 0.1μM α-DTX application (middle) and 0.5mM 4-AP+0.1μM α-DTX application in the rapidly adapting neuron. Right panel shows the first action potentials after TTX application (control), α-DTX application, and α-DTX+4-AP application. (D), Changes in the number of spikes (a), resting membrane potential (b), duration of half amplitude of action potential and DDP in response to α-DTX in the absence and presence of 4-AP. Values show mean ± S.E.M. *, $P<0.05$ (n=6) versus control, #, $P<0.05$ (n=6) versus after 4-AP application.

Fig. 5. Effect of TEA in the absence or presence of α-DTX on I_K. (A), A typical effect of TEA (2mM) application in the absence of α-DTX (0.1μM) on I_K (a) and summary of TEA (2mM) on the normalized I-V relationship (b). The percent inhibition of I_K by TEA (2mM) (c). Values shows mean ± S.E.M. *, $P<0.05$ (n=5) versus control. (B), A typical effect of TEA (2mM) in the presence of α-DTX (0.1μM) (a) and summary of TEA (2mM) and α-DTX (0.1μM) effect on the normalized I-V relationship (b). The percent inhibition of I_K by α-DTX (0.1μM) in the absence (□) and presence (■) of TEA (2mM).

Values show mean ± S.E.M. *, P<0.05 (n=5) versus control, #, P<0.05 versus after TEA application.

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 (210pA) after 1μM TTX (control, left), 0.1μM α-DTX (middle) and 2mM TEA+0.1μM α-DTX in the slowly adapting type TG neuron. Right panel shows the 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 and duration of depolarizing phase of action potentials (DDP)(d) in response to α-DTX in absence and presence of TEA. (C), The action potentials were induced by a 3T current injection (180pA) after 1μM TTX (control, left), 0.1μM α-DTX (middle) and 2mM 4-AP+0.1μM α-DTX application in the rapidly adapting type TG neuron. Right panel shows the 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 and DDP 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.

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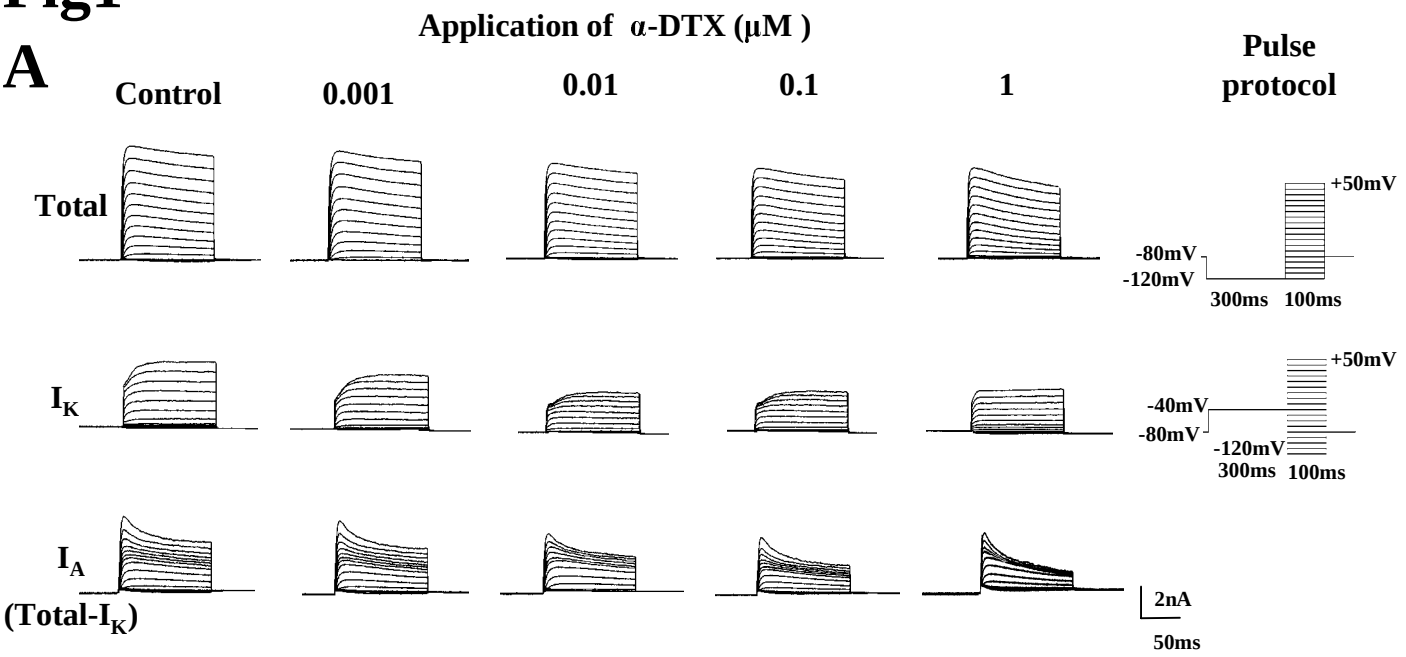
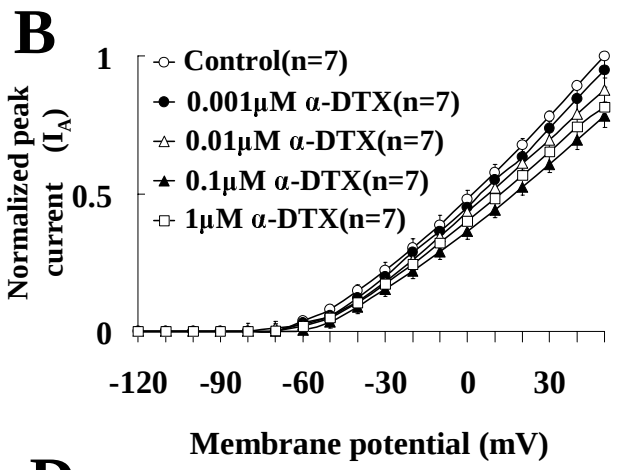
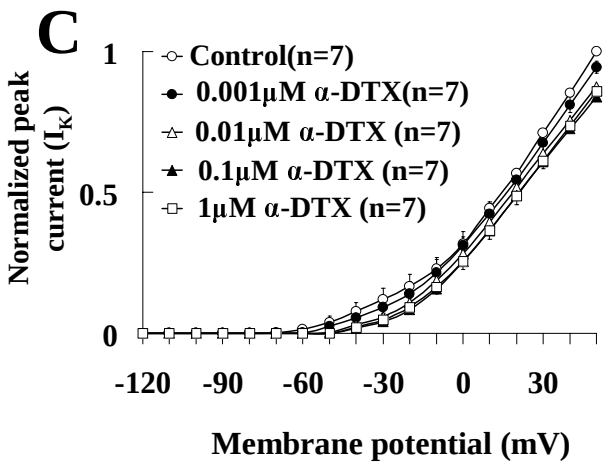
Fig1







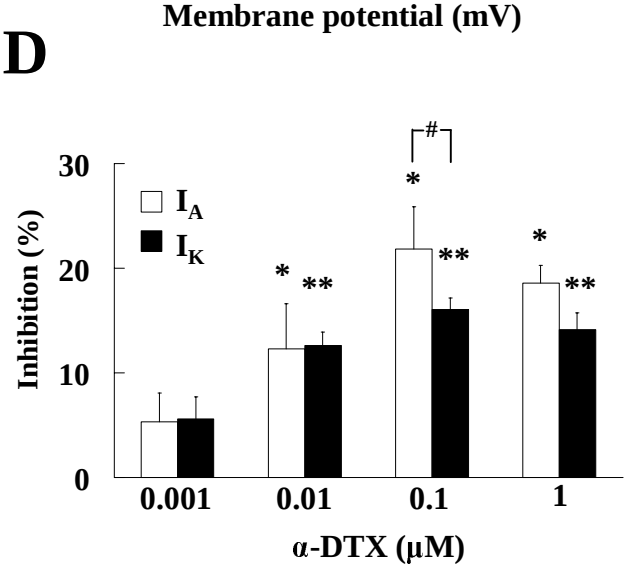


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Fig2

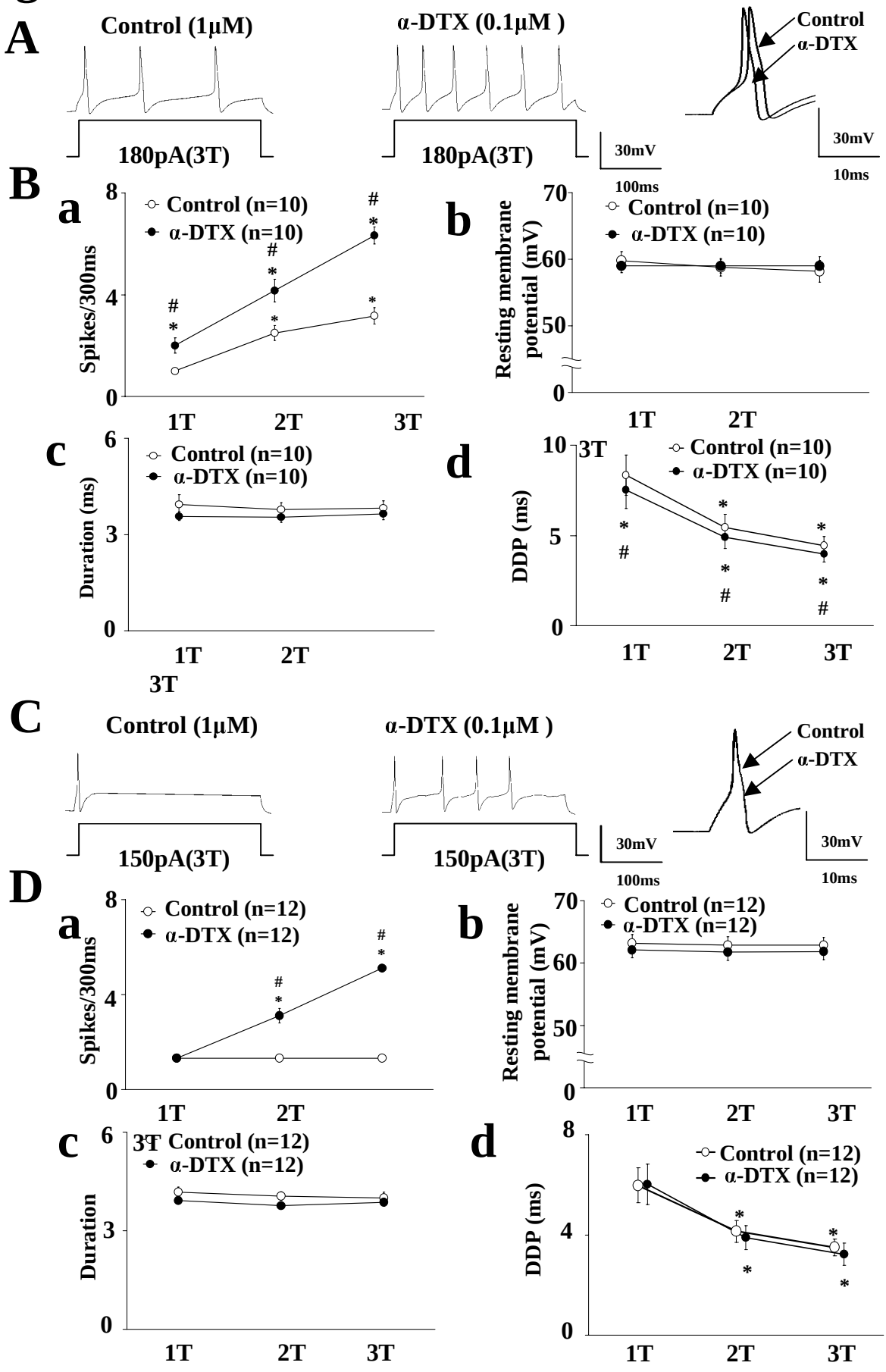

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Fig3

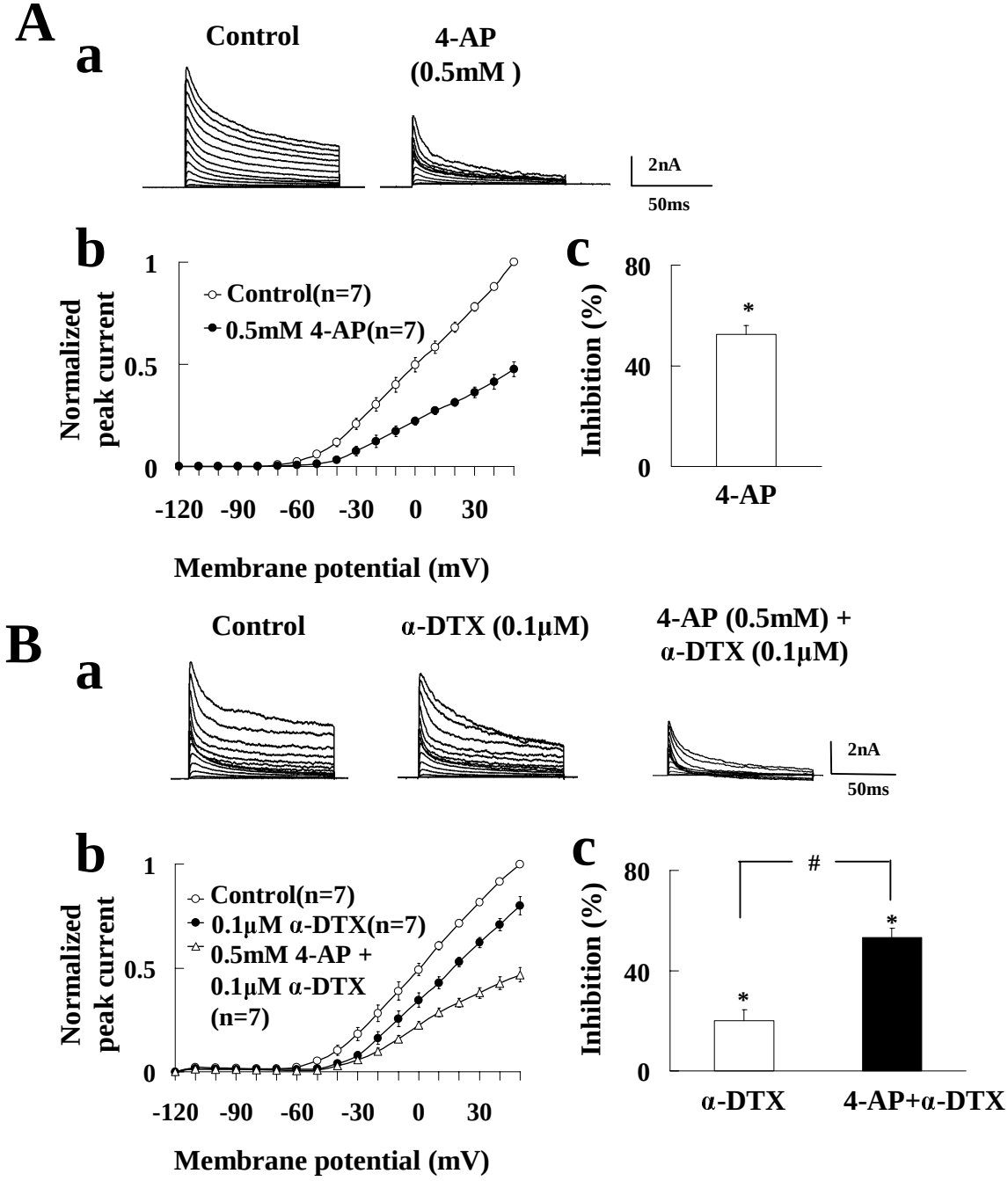

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Fig4

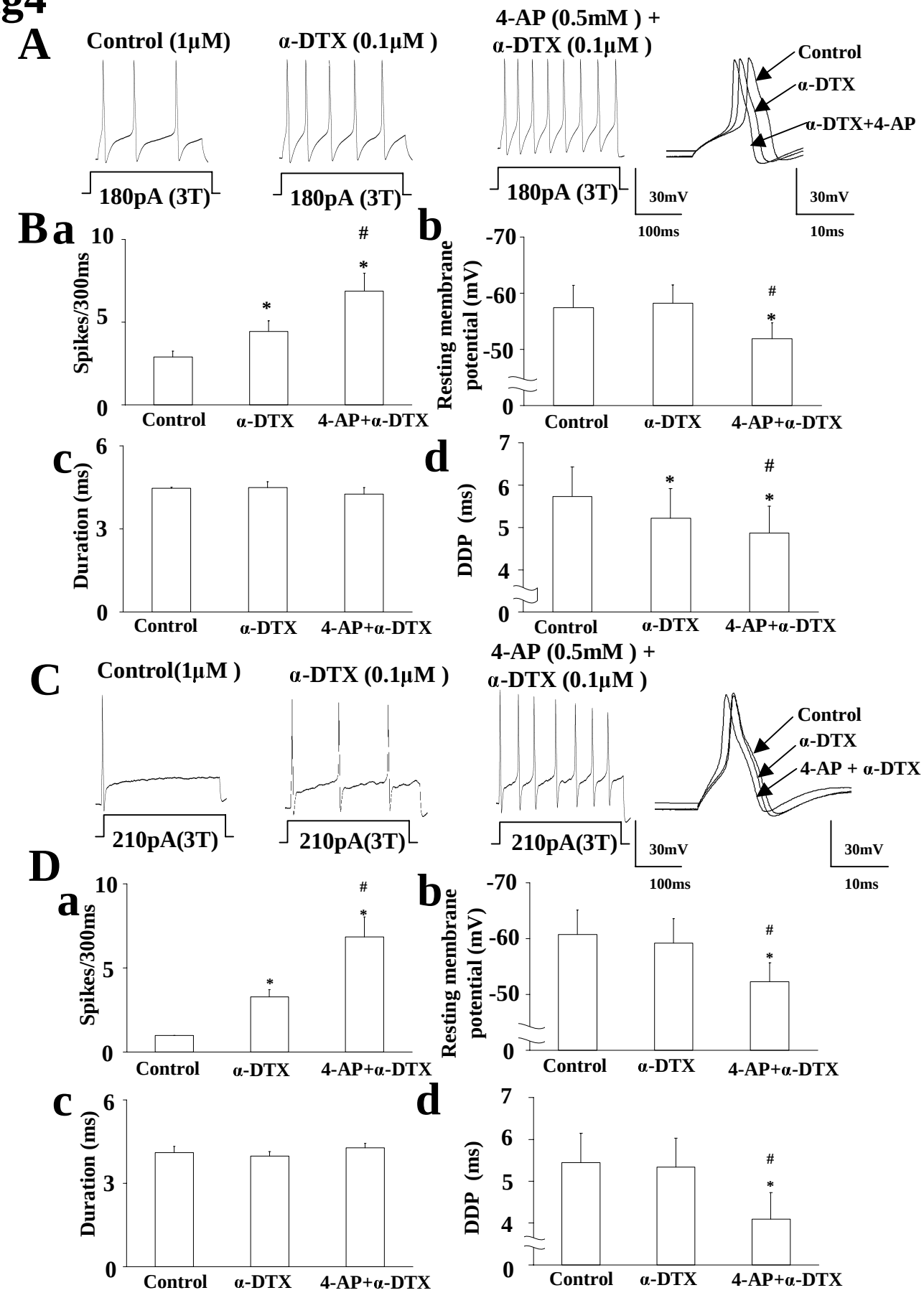

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Fig5

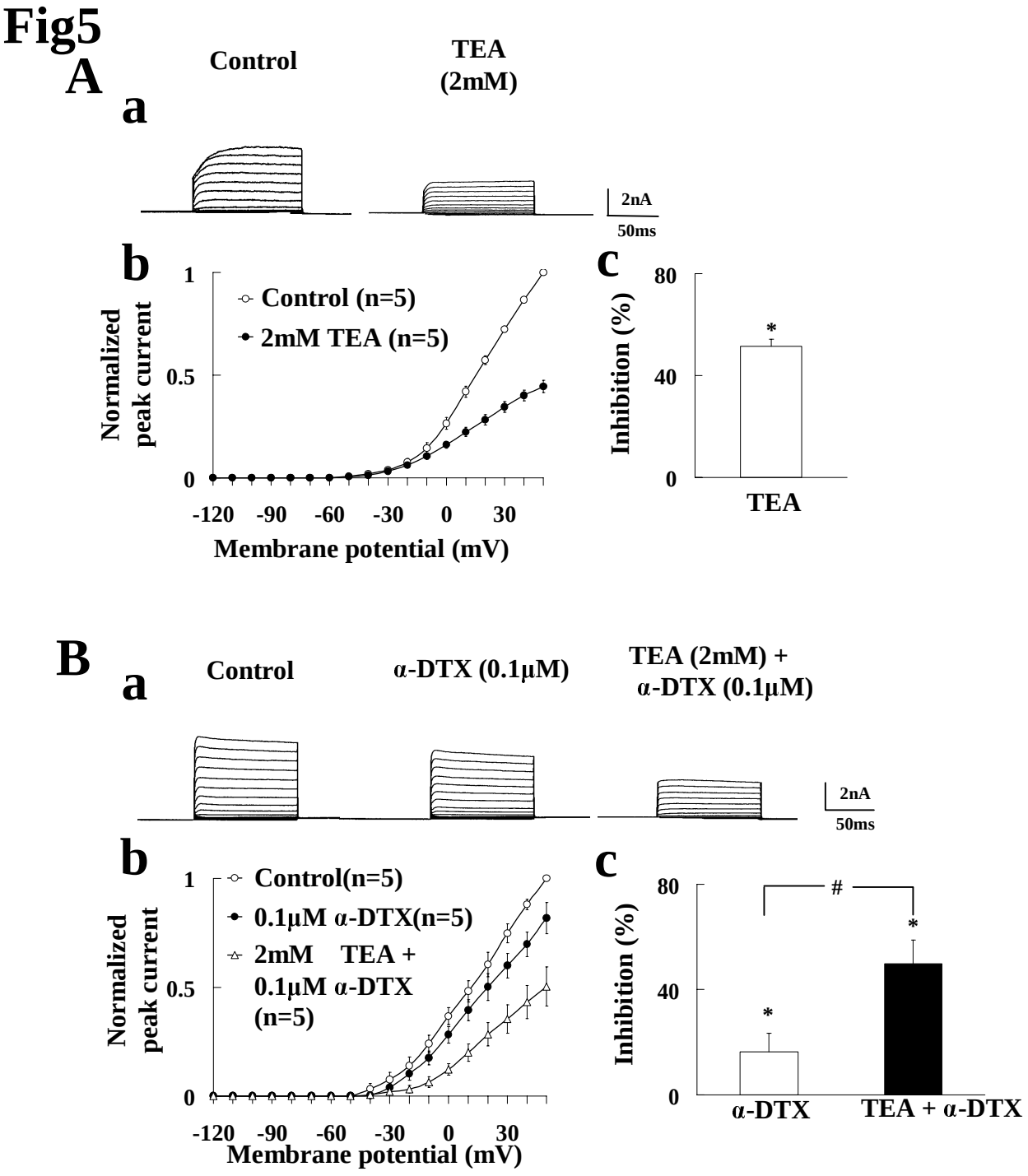

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Fig6