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RAPID REPORT

Dipeptidyl-peptidase-like proteins confer high sensitivity to the scorpion toxin AmmTX3 to Kv4-mediated A-type K^+ channels

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Key points

- AmmTX3, a member of the α-KTX15 family of scorpion toxins, efficiently and specifically blocks the subthreshold-operating A-type K^+ current in cerebellar granule neurons from wild-type mice but not in neurons from mice lacking the Kv4 channel-associated protein DPP6.
- In heterologous cells high-affinity blockade of Kv4.2 and Kv4.3 channels by AmmTX3 requires the presence of the associated proteins DPP6 and DPP10.
- These results validate AmmTX3 as a specific blocker of Kv4 channels in CNS neurons and explain the discrepancy between previous observations in neurons and heterologous cells.
- They contribute a powerful tool to investigate the physiological role of A-type K^+ currents, believed to be important in dendritic integration and plasticity and to be involved in a number of diseases.
- Our results demonstrate that, in addition to changing the kinetics and voltage dependence of Kv4 channel complexes, DDP-like Kv4-associated proteins also affect their pharmacological profile.

Abstract K^+ channels containing Kv4.2 and Kv4.3 pore-forming subunits mediate most of the subthreshold-operating somatodendritic A-type K^+ current in CNS neurons. These channels are believed to be important in regulating the frequency of repetitive firing, the backpropagation of action potential into dendrites, and dendritic integration and plasticity. Moreover, they have been implicated in several diseases from pain to epilepsy and autism spectrum disorders. The lack of toxins that specifically and efficiently block these channels has hampered studies aimed at confirming their functional role and their involvement in disease. AmmTX3 and other related members of the α-KTX15 family of scorpion toxins have been shown to block the A-type K^+ current in cultured neurons, but their specificity has been questioned because the toxins do not efficiently block the currents mediated by Kv4.2 or Kv4.3 subunits expressed in heterologous cells. Here we show that the high-affinity blockade of Kv4.2 and Kv4.3 channels by AmmTX3 depends on the presence of the auxiliary subunits DPP6 and DPP10. These proteins are thought to be components of the Kv4 channel complex in neurons and to be important for channel expression

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in dendrites. These studies validate the use of AmmTX3 as a blocker of the Kv4-mediated A-type K^+ current in neurons.

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Abbreviations CGN, cerebellar granular neuron; DPP, dipeptidyl peptidase-like protein; I_{SA}, subthreshold-operating A-type K^+ current; I_{to}, transient outward potassium current; KChIP, K^+ channel-interacting protein; KO, knockout; MSN, medium spiny neuron; TEA, tetraethylammonium; TTX, tetrodotoxin; WT, wild type.

Introduction

The availability of specific blockers has greatly advanced our ability to study the contribution of individual ion channels to cellular and tissue function and pathology. K^+ channels containing pore-forming subunits of the Kv4 subfamily (Kv4 channels) are thought to mediate most of the subthreshold-operating somatodendritic transient or A-type K^+ current in neurons (also known as I_{SA}) and the transient outward potassium current (I_{to}) in the heart (Jerng *et al.* 2004; Maffie & Rudy, 2008; Niwa & Nerbonne, 2010). There are three Kv4 members known, of which two, Kv4.2 and Kv4.3, are the main components of Kv4 channels in the CNS (Serodio & Rudy, 1998; Rhodes *et al.* 2004; Strassle *et al.* 2005). In neurons, Kv4 channels are thought to regulate the frequency of repetitive firing, the backpropagation of the action potential into the dendritic tree and dendritic integration and plasticity (Hoffman *et al.* 1997; Cai *et al.* 2004; Jerng *et al.* 2004; Magee & Johnston, 2005; Maffie & Rudy, 2008). They have recently been implicated in mediating a number of important functions such as the compartmentalized changes in dendritic branch excitability that are important in learning (Losonczy *et al.* 2008) and the differential Ca^{2+} elevation in the dendrites of D1 and D2-expressing striatal medium spiny neurons (MSNs) (Day *et al.* 2008). However, it has been difficult to directly demonstrate that the currents responsible for these functions are Kv4-mediated due to the lack of adequate channel blockers specific for these channels.

One group of toxins that has been investigated is the α-KTX15 family which now includes 6 highly homologous peptides found in four species of scorpion venoms (Aa1; AaTX1, AaTX2; AmmTX3 BmTX3 and Discrepin) (Vacher *et al.* 2002, 2004, 2006; D'Suze *et al.* 2004; Martin-Eauclaire & Bougis, 2012). These toxins were shown to block the I_{SA} of cultured cerebellar granular neurons (CGNs) and MSNs in the striatum, both of which have been shown to be mediated by channels containing Kv4 pore-forming subunits (Serodio & Rudy, 1998; Tkatch *et al.* 2000; Rhodes *et al.* 2004; Strassle *et al.* 2005; Amarillo *et al.* 2008). An active iodinated preparation of BmTX3 (I^{125}-BmTX3) was found to bind with high affinity to specific sites in rat brain strikingly similar to those expressing Kv4 proteins. The binding was highly specific for α-KTX15 toxins. Other members of the α-KTX15 family, but not any other peptidyl neurotoxins, displaced I^{125}-BmTX3 binding (Vacher *et al.* 2006).

In spite of these observations, α-KTX15 toxins have not been used in the field, largely because of concerns about their specificity arising from the inability to demonstrate that they efficiently block the currents mediated by Kv4.2 or Kv4.3 subunits expressed in heterologous cells (Vacher *et al.* 2006). While heterologously expressed Kv4.2 and Kv4.3 currents (but not other rapidly inactivating Kv currents such as Kv1.4 and Kv3.4) were blocked by the toxins, they were much less sensitive than the Kv4-like current in neurons (Vacher *et al.* 2006).

The Kv4 channel complex that mediates the somatodendritic I_{SA} in neurons includes, in addition to pore-forming Kv4 proteins, at least two classes of accessory subunits: K^+ channel-interacting proteins (KChIPs) and the dipeptidyl peptidase-like proteins (DPPs) DPP6 and DPP10 (An *et al.* 2000; Nadal *et al.* 2003; Jerng *et al.* 2004; Zagha *et al.* 2005; Maffie & Rudy, 2008). We hypothesized that auxiliary subunits may account for the differences between the effects of α-KTX15 toxins on Kv4 channels in heterologous cells and in neurons. DPPs are particularly good candidates because they interact with the transmembrane domains of Kv4 proteins and are likely to alter the extracellular structure of Kv4 channel complexes (Ren *et al.* 2005; Zagha *et al.* 2005; Dougherty *et al.* 2009). We took advantage of the availability of DPP6 knockout (KO) mice to explore the possibility that DPP6 increases the α-KTX15 toxin sensitivity of the Kv4-mediated I_{SA}. We found that AmmTX3 efficiently blocks the I_{SA} recorded in CGNs from wild-type (WT) but not DPP6 KO mice. These experiments suggest that DPPs are necessary for high-affinity block of the I_{SA} by α-KTX15 toxins. This conclusion is supported by experiments in heterologous cells.

Methods

All experiments were carried out in accordance with the NIH *Guide for the Care and Use of Laboratory Animals* and were approved by the New York University School of Medicine Animal Care and Use Committee.

Drugs

Recombinant AmmTX3 and BmTX3 were produced in *Escherichia coli* as previously described (Vacher *et al.* 2002, 2004).

Patch-clamp recording from heterologous mammalian cells

Electrophysiological recording from heterologous mammalian cells was carried out as previously described (Zagha *et al.* 2005). CHO-K1 cells were transfected with the appropriate plasmids using X-tremeGENE 9 DNA Transfection Reagent (Roche Diagnostics GmbH, Mannheim, Germany). Kv4.2- or Kv4.3-mediated currents were recorded 24 h later using the whole-cell configuration of the patch-clamp method with a pipette (intracellular) solution containing (in mM): 144 mm potassium gluconate, 0.2 EGTA, 3 $MgCl_2$, 10 Hepes, 4 MgATP, and 0.5 NaGTP (pH adjusted to 7.2 with KOH). During recording, cells were perfused with ACSF (see Patch-clamp recordings from CGNs section below). Bovine Serum Albumin (BSA; 0.2%; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was added to toxin-containing solutions to prevent toxin adsorption to plastic. Series resistance ($R_s = 2$–12 MΩ) was compensated to yield a compensated access resistance of less than 5 MΩ. Currents were filtered at 5 kHz and sampled at 10 kHz. The I_{SA} was isolated using the prepulse protocol described in Amarillo *et al.* (2008) by subtracting the currents recorded during depolarizing steps (Vp; from −90 to +70 mV) preceded by a 1 s prepulse to −40 mV from the currents obtained during depolarizing steps preceded by a 1 s prepulse to −110 mV. All measurements were taken at room temperature and results are expressed as means ± SEM. All reagents were supplied by Sigma (Sigma-Aldrich, St Louis, MO, USA), unless otherwise specified.

Patch-clamp recordings from CGNs

Acute brain slices from 15- to 21-day-old DPP6 KO mice and WT littermates (Clark *et al.* 2008; Sun *et al.* 2011) were prepared using standard techniques as previously described in Amarillo *et al.* (2008). Mice were anaesthetized with intraperitoneal injection of pentobarbital (100 mg (kg body weight^{-1})) and decapitated. The brain was quickly removed and immersed in ice-cold oxygenated artificial CSF (ACSF) containing the following (in mM): 87 NaCl, 75 sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1 $CaCl_2$, 2 $MgSO_4$ and 10 glucose. Slices (300 μm thick) were cut on a vibratome (Vibratome 3000 Plus, TED PELLA, Redding, CA, USA) and incubated in a holding chamber at 32–35°C for approximately 30 min followed by continued incubation at room temperature prior to electrophysiological recording, at which point, slices were transferred to a recording chamber and perfused at room temperature with ACSF (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 1 $MgSO_4$, 2 $CaCl_2$, and 10 glucose, containing, except when otherwise noted 5 mM tetraethylammonium (TEA) and 0.5 μM tetrodotoxin (TTX, Tocris Bioscience, Bristol, UK). Voltage clamp recordings were performed from CGNs in the anterior lobules of cerebellum using the whole-cell configuration of the patch-clamp technique. Patch electrodes (5–8 MΩ) were filled with potassium gluconate-based internal solution as in heterologous cell recording. Membrane potentials were corrected for a −10 mV junction potential. Currents were recorded using an Axopatch 200B amplifier (Axon Instruments, Molecular Devices, Sunnyvale, CA, USA), low pass filtered at 5 kHz, digitized at 16-bit resolution (Digidata 1322A; Axon Instruments) and sampled at 10 kHz. Data obtained from a given cell were rejected if series resistance (R_s) was larger than 50 MΩ or changed by >20% during the course of the experiment.

Results

AmmTX3 efficiently blocks the I_{SA} of cerebellar granule neurons (CGNs) from WT but not DPP6 KO mice

CGNs have a large I_{SA}, consistent with prominent expression of Kv4.2 and Kv4.3 mRNAs and proteins (Serodio & Rudy, 1998; Rhodes *et al.* 2004; Strassle *et al.* 2005). A large number of observations including detailed electrophysiological comparison and gene ablation methods have demonstrated that the transient current in CGNs is Kv4 mediated (e.g. Shibata *et al.* 2000; Amarillo *et al.* 2008; Nadin & Pfaffinger, 2010). CGNs are also among the few neuronal populations where the patterns of expression of the various molecular components of Kv4 channels have been analysed in detail (Serodio & Rudy, 1998; Rhodes *et al.* 2004; Strassle *et al.* 2005; Zagha *et al.* 2005). Moreover, compared to other neurons, CGNs are electrotonically compact due to their small size (~5 μm) and the short length of dendritic processes, allowing good voltage control for whole-cell recording (Cull-Candy *et al.* 1989). This is particularly important in the case of Kv4 channels since they are highly enriched in dendrites (Hoffman *et al.* 1997; Rhodes *et al.* 2004; Clark *et al.* 2008). We therefore selected these neurons to investigate the pharmacological properties of the neuronal I_{SA} in acute slices from WT and DPP6 KO mice.

In addition to Kv4 proteins, CGNs prominently express Kv3.1 subunits (Weiser *et al.* 1995), which mediate a very large slow inactivating component of the K^+ current (Amarillo *et al.* 2008). We compared the effects of 0.5 μM AmmTX3 on the transient and sustained components of the outward current. At this concentration, AmmTX3

blocked the transient component of the current nearly completely ($n = 3$), as previously observed in cultured neurons, without affecting the sustained component ($4 \pm 6\%$ block, $n = 3$) (Fig. 1*A*).

To quantify the effects of AmmTX3 on the I_{SA} it is necessary to isolate this component from the total current. The classic strategy for the isolation of native I_{SA} from neurons uses prepulse protocols to inactivate the A-type K^+ channels. The I_{SA} is then obtained by subtraction of the current remaining after the prepulse from the total current (e.g. Connor & Stevens, 1971; Cull-Candy *et al.* 1989). However, in slices from mice older than P7, the classic protocol is not useful for isolating the I_{SA} from CGNs, except during relatively small depolarizations, because the prepulse also inactivates a significant amount of the slowly inactivating component (Cull-Candy *et al.* 1989; Amarillo *et al.* 2008). Therefore, to facilitate the isolation of the I_{SA}, we followed the strategy of Amarillo *et al.* (2008), which utilized relatively low concentrations of TEA (5 mM), a drug that does not block Kv4 channels but completely blocks Kv3 channels (Coetzee *et al.* 1999). This concentration of TEA blocked a major portion of the total

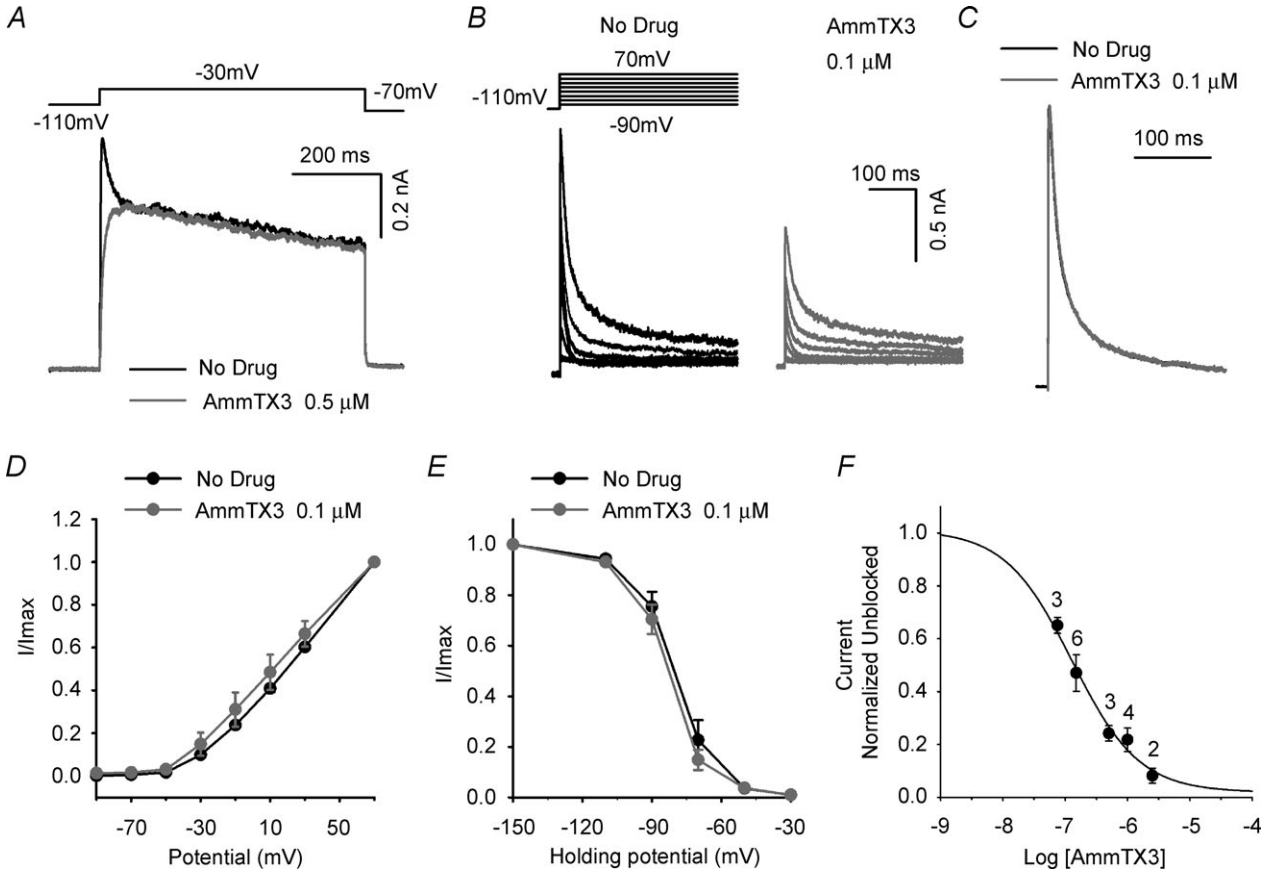

Figure 1. Specific block of the A-type K^+ current by AmmTX3 in CGNs
A, AmmTX3 (0.5 μM) blocked the transient but not the sustained component of the outward current of CGNs from WT mice in acute slices. Shown are the currents recorded before (black) and after (grey) the application of 0.5 μM AmmTX3 during a depolarization (V_p) to −30 mV following a 1 s prepulse to −110 mV from a holding potential (V_h) of −70 mV in a solution containing 0.5 μM TTX. *B*, block of the isolated I_{SA} by 0.1 μM AmmTX3. Shown is the I_{SA} isolated using the prepulse protocol from CGNs recorded in the presence of 5 mM TEA and 0.5 μM TTX before (black) and after (grey) the application of 0.1 μM AmmTX3 during depolarizations from −90 to 70 mV. *C*, the I_{SA} recorded at $Vp = 50$ mV in the presence of 0.1 μM AmmTX3 (grey; average of 4 traces) is scaled and superimposed on the current recorded at the same membrane potential before toxin application (black; average of 4 traces). *D*, the fraction of I_{SA} blocked by AmmTX3 is the same at different membrane potentials. The relative peak current (I/I_{max}) of the I_{SA} remaining after application of 0.1 μM AmmTX3 as a function of membrane potential is superimposed on the I/I_{max} of the I_{SA} recorded before the application of AmmTX3 ($n = 3$). *E*, the voltage dependence of steady-state inactivation of I_{SA} does not change after application of 0.1 μM AmmTX3. To obtain the steady-state inactivation curve the I_{SA} was elicited by step depolarizations to 30 mV, preceded by a 1 s prepulse from −150 to −30 mV from a holding potential of −70 mV ($n = 3$). *F*, dose–response curve for CGNs I_{SA} block by AmmTX3. The curve was fitted with an $IC_{50} = 0.129$ μM and $k = -0.8$ ($n = 2, 3, 6, 3, 4, 2$ at 0; 0.075; 0.15: 0.5, 1 and 2.5 μM AmmTX3, respectively). Error bars are SEM.

outward current in CGNs, leaving a highly enriched I_{SA} that could be isolated using a prepulse protocol (Fig. 1*B*; Amarillo *et al.* 2008).

AmmTX3 blocked the isolated I_{SA} in CGNs from WT control mice; 0.1 μM blocked about 50% of the current (Fig. 1*B*), while 0.5 μM blocked the current nearly completely (Fig. 2*A*). A dose–response curve is shown in Fig. 1*F*. The toxin blocked the current in WT CGNs with a half-maximal blocking concentration of approximately 0.1 μM, close to the IC_{50} reported for AmmTx3 and other α-KTX15 toxins in cultured neurons (Vacher *et al.* 2002). The voltage dependence of activation (Fig. 1*D*), steady-state inactivation (Fig. 1*E*) and the kinetics (Fig. 1*C*) of partially blocked currents were identical to those measured in the absence of toxin. These results suggest that AmmTx3 is not a gating modifier as is the case with heteropodatoxins, spider toxins that block Kv4.2 currents in a voltage-dependent manner, producing less block at more positive potentials, and changing the channels voltage dependence and kinetics (Sanguinetti *et al.* 1997). Instead, AmmTX3 is likely to work as a pore blocker, since the toxin contains the functional dyad (K27 and Y36) characteristic of pore-blocking potassium channel-specific toxins (Dauplais *et al.* 1997). In fact BmTx3 lacking the last two C-terminal residues, including Y36, does not block the A-type currents (Vacher *et al.* 2003).

The time course of block of the I_{SA} in CGNs was relatively slow with a time of half-block of around six minutes (see Fig. 2*C*), which is due, at least in part, to slow diffusion of the toxin into the tissue, since in cultured heterologous cells (Fig. 3*C*) and in cultured neurons (Vacher *et al.* 2006) the time course was at least three times faster.

In contrast to the results in WT mice, in CGNs from DPP6 KO mice the currents were only weakly affected by the toxin at five times the IC_{50} observed in WT neurons (Fig. 2*A*). At 0.5 μM AmmTX3, a concentration that blocked >80% of the isolated I_{SA} in WT neurons, only $12 \pm 8\%$ ($n = 5$) of the current was blocked in neurons from DPP6 KO mice (Fig. 2*B*). This suggests that the sensitivity of DPP6-lacking channels is about 20-fold lower than that of channels containing DPP6.

This study is focused on the effects of AmmTX3 since this is the most readily available α-KTX15 toxin, but the results are likely to apply to other members of the family since they all are structurally extremely similar and they all compete for I^{125}-BmTX3 binding to brain membranes (Vacher *et al.* 2006). Pilot experiments with BmTX3 support this conclusion. At 0.1 μM, BmTX3 blocked $44 \pm 2\%$ ($n = 3$) of the I_{SA} in WT CGNs and $19 \pm 3\%$ ($n = 3$) of I_{SA} in CGNs from DPP6 KO mice.

The results suggest that DPPs are necessary for high sensitivity of Kv4 channels to α-KTX15 toxins. This hypothesis was further tested in experiments in heterologous expression systems.

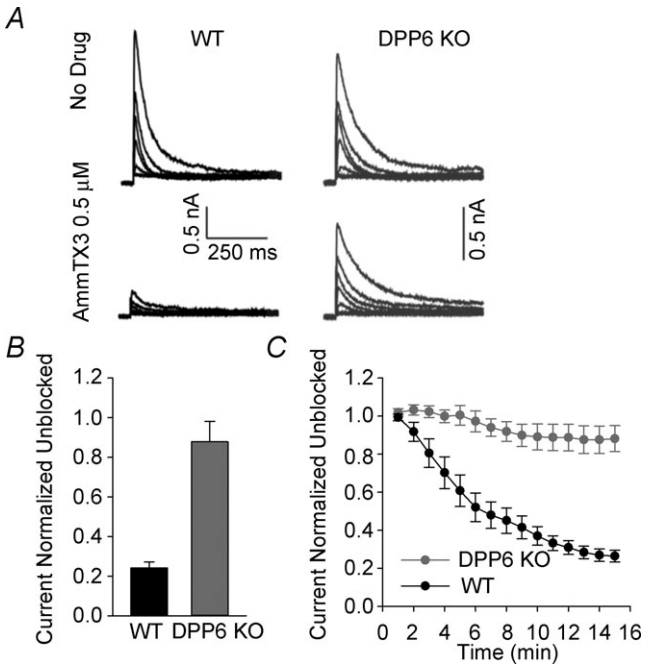

Figure 2. AmmTX3 blocks the I_{SA} in CGNs of wild-type (WT) mice more efficiently than in CGNs of DPP6 knockout (KO) mice

A, block of the isolated I_{SA} by 0.5 μM AmmTX3 in WT and DPP6 KO mice. Shown is the I_{SA} isolated using the prepulse protocol described in Methods from CGNs from WT (black) and DPP6 KO (grey) mice recorded in the presence of 5 mM TEA and 0.5 μM TTX, before (upper traces) and after (lower traces) the application of 0.5 μM AmmTX3 during depolarizations to −90 to 70 mV. *B*, population data showing the block of the I_{SA} recorded during a depolarization to 50 mV by 0.5 μM AmmTX3 in CGNs from WT (black; $n = 3$) and DPP6 KO (grey; $n = 3$) mice ($P < 0.005$, *t* test). *C*, time course of block of the I_{SA} recorded at $V_p = 50$ mV from DPP6 KO (grey; $n = 5$) and WT (black; $n = 4$) mice following the application of 0.5 μM AmmTX3. Error bars are SEM.

DPPs increase the sensitivity to AmmTX3 of Kv4 channels expressed in heterologous cells

It could be argued that the I_{SA} in CGNs from KO mice is not Kv4-mediated due to compensatory mechanisms. This is unlikely because, in spite of the changes in current properties produced by the loss of DPP6, the current in neurons from KO mice resembles Kv4 currents more than the transient K^+ current mediated by other pore-forming subunits (Sun *et al.* 2011). To independently test the hypothesis that DPPs increase the sensitivity of Kv4 channels to AmmTx3 we tested their influence on Kv4 channels expressed in heterologous expression systems. Kv4 subunits require KChIP or DPP accessory proteins for efficient expression in heterologous cells (Maffie & Rudy, 2008). We compared the sensitivity of Kv4 channels containing Kv4.2 and KChIP1 proteins with those of

channels containing Kv4.2 and DPP6 proteins in CHO cells.

The current in CHO cells expressing Kv4.2 and KChIP1 proteins was weakly blocked by 0.5 μM AmmTX3, similar to the block of the I_{SA} in CGNs from DPP6 KO mice (Fig. 3*A* and *B*). On the other hand, in the presence of the DPP6 splice variant DPP6S the currents were efficiently blocked to a similar degree as the I_{SA} in CGNs from WT mice, independently of whether KChIP was present or not (Fig. 3*A* and *B*), supporting the notion that DPP6 proteins increase the affinity of Kv4 channels for AmmTX3. Block of DPP6S-containing Kv4.2 channels was reversible; a considerable portion of the current could be recovered following washout of the toxin (Fig. 3*C*).

Several neuronal populations in the CNS express Kv4.3 instead of Kv4.2 subunits (Serodio & Rudy, 1998; Rhodes *et al.* 2004; Strassle *et al.* 2005). Often neurons expressing Kv4.3 have the DPP6-related subunit DPP10 (Zagha *et al.* 2005). We investigated the AmmTX3 sensitivity of Kv4.3 channels with or without the DPP10 splice variant DPP10a to determine whether the observed effects of DPP6 are specific to DPP6 or are a general property of DPPs. As

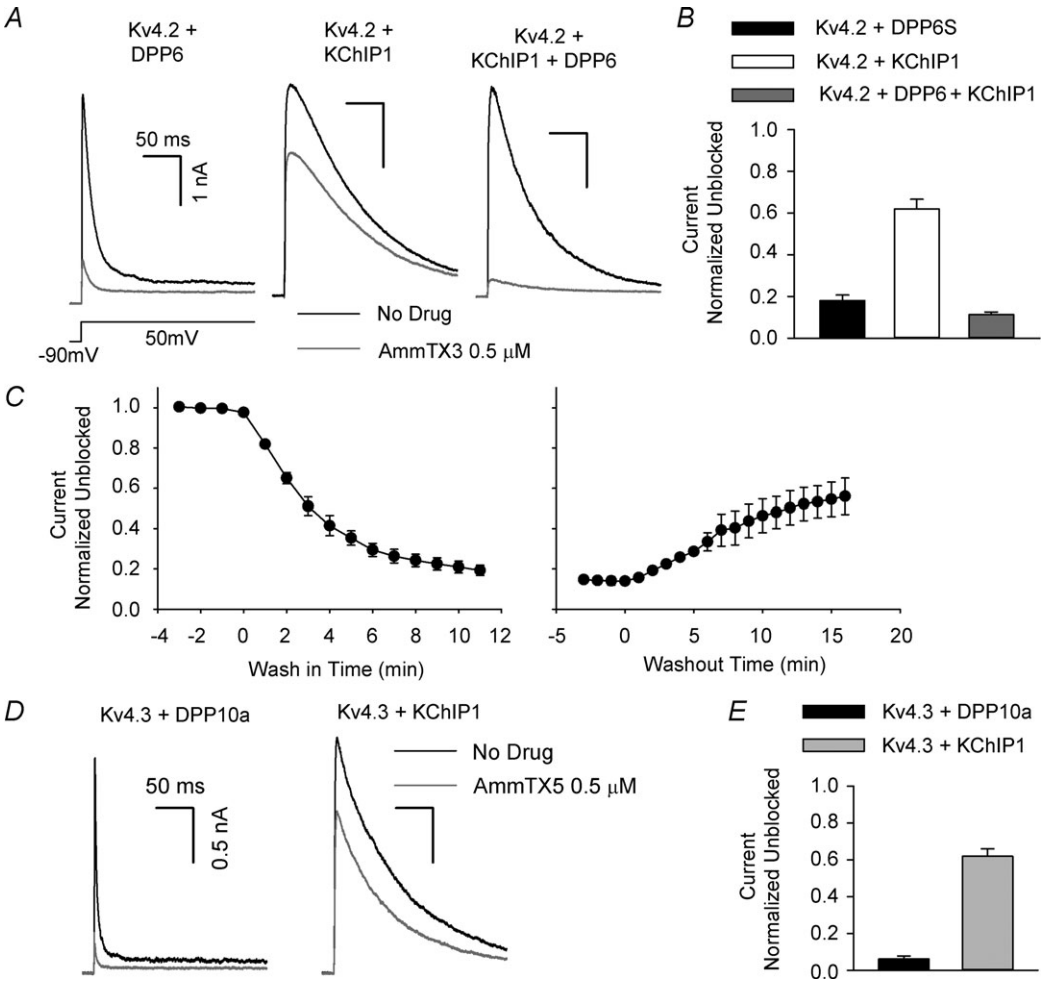

Figure 3. DPPs increase the efficiency of AmmTX3 block of Kv4 currents expressed in CHO cells
A, AmmTX3 (0.5 μM) blocks the Kv4.2 current expressed in CHO cells co-transfected with DPP6S (left) or with DPP6S + KChIP1 (right) nearly completely, but only partially blocks the current expressed in CHO cells co-transfected with KChIP1 (middle) in the absence of DPP6. Shown are the currents recorded before (black) and after (grey) the application of 0.5 μM AmmTX3 during a depolarization to 50 mV following a 1 s pre-pulse to −90 mV from a holding potential of −70 mV. *B*, population data. $n = 7$, 5 and 6 for Kv4.2 + DPP6S, Kv4.2 + KChIP1 and Kv4.2 + DPP6S + KChIP1, respectively. *C*, time course of block by 0.5 μM AmmTX3 (left; $n = 3$) and washout (right; $n = 3$) of the I_{SA} recorded at $V_p = 50$ mV from CHO cells transfected with Kv4.2 and DPP6S. *D*, AmmTX3 (0.5 μM) blocks the Kv4.3 current expressed in CHO cells co-transfected with DPP10a nearly completely (left), but only partially blocks the current expressed in CHO cells co-transfected with KChIP1 in the absence of DPP10a (right). Shown are the currents recorded before (black) and after (grey) toxin application. $V_h = -90$ mV, $V_p = 50$ mV. *E*, population data $n = 6$, 6 for Kv4.3 + DPP10a, Kv4.3 + KChIP1 respectively. Error bars are SEM.

shown in Fig. 3*D* and *E*, DPP10a increased the sensitivity to AmmTX3 of Kv4.3 channels expressed in CHO cells, similar to the effect of DPP6S on Kv4.2 channels.

Discussion

Our studies in acute slices have confirmed previous observations in cultured neurons showing that AmmTX3 efficiently blocks the I_{SA} without affecting the sustained component of the K^+ current, which in CGNs is largely mediated by Kv3 channels (Weiser *et al.* 1995). Furthermore, our studies in native neurons and in heterologous cells demonstrate that the DPPs DPP6 and DPP10 increase the sensitivity of Kv4 channels to the toxin, providing an explanation for the discrepancies between native and reconstituted channels previously noted by Vacher *et al.* (2006). While the mechanisms of channel block by these toxins and the mechanisms by which DPPs affect toxin sensitivity remain to be investigated, this study clarifies a key issue that had prevented many in the field from confidently utilizing this toxin and demonstrates that AmmTX3 is a useful tool to study the role of native Kv4 channels *in vitro* and *in vivo*.

At the same time, these findings provide additional evidence that native I_{SA} channels in CNS neurons contain DPP proteins. Association between Kv4 channels and DPP6 was first discovered when it was observed that DPP6 co-purifies with Kv4.2 proteins from detergent extracts of brain membranes. Co-expression of Kv4.2 or Kv4.3 and DPP6 or DPP10 in heterologous cells produces changes in voltage dependence and kinetics, resulting in currents that resemble more closely native currents in neurons (Nadal *et al.* 2003; Zagha *et al.* 2005; reviewed in Jerng *et al.* 2004; Maffie & Rudy, 2008). Evidence that native Kv4 channels in neurons have DPPs also includes the observation that the distribution of DPP6 proteins in brain closely resembles the distribution of Kv4.2 proteins (Clark *et al.* 2008) and that ablation of DPP6 by siRNA treatment or gene knockout results in changes in the voltage dependence and kinetic properties of the native currents largely consistent with the observations of the effects of DPPs on channels expressed in heterologous cells (Kim *et al.* 2008; Nadin & Pfaffinger, 2010; Sun *et al.* 2011). The present study demonstrates that DPPs also affect the pharmacological properties of the channels. AmmTX3 blocks native neuronal I_{SA} channels and DPP-containing Kv4 channels in heterologous cells with similar affinity, strongly suggesting that inclusion of DPPs in heterologous cells reconstitutes the native channel's pharmacology. The availability of a toxin with complex selective effects may facilitate future experiments examining the composition of Kv4 channels.

Interestingly, the affinity of the *slo*-mediated Ca^{2+}-activated K^+ channel ($BKCa^{2+}$ or maxi-K^+) for another scorpion toxin, charybdotoxin (ChTX), is also dependent on the presence of auxiliary subunits in the channel complex. Garcia and colleagues have demonstrated a large increase in the channel's affinity for ChTX when the *slo* pore-forming subunit is co-expressed with its β-subunit (Hanner *et al.* 1997).

AmmTx3 likely binds to the extracellular surface of the Kv4 pore-forming subunits since it also blocks, although with lower affinity, channels containing only these subunits (Vacher *et al.* 2006) or channels containing KChIP subunits, which are intracellular proteins (this study). This implies that the structure of the extracellular face of the Kv4 proteins is changed upon association with DPPs. High-resolution solid-state NMR spectroscopy, has shown that the high-affinity binding of the scorpion toxin kaliotoxin to a chimaeric K^+ channel (KcsA-Kv1.3) is associated with significant structural rearrangements (induced-fit) in both molecules. Thus, the structural flexibility of the K^+ channel and the toxin represents an important determinant for the high specificity of toxin–K^+ channel interactions (Lange *et al.* 2006). Structural studies of the effects of DPPs on Kv4 proteins will be helpful to understand the molecular mechanisms by which α-KTX15 toxins block Kv4 channels.

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The experiments were carried out in the Department of Physiology and Neuroscience, Smilow Neuroscience Program, NYU School of Medicine, New York, NY, USA and in the CRN2M, CNRS UMR 7286, Aix-Marseille University, Faculté de Médecine Nord, Marseille, France. The conception and design of this study was by B.R., J.M., E.D. and M.-F.M.-E. Experiments were carried out by J.M., P.E.B. and E.D. The data were analysed and interpreted by J.M., E.D. and B.R. All authors contributed to writing the manuscript. The final version of the manuscript was read and approved by all the authors.

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