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Dendrotoxin: a selective blocker of a non-inactivating potassium current in guinea-pig dorsal root ganglion neurones*

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Abstract. 1. The voltage clamp technique was used to study the effects of dendrotoxin (DTX) on outward potassium currents in internally perfused dorsal root ganglion neurones of guinea-pig. Sodium currents were eliminated by tetrodotoxin (TTX, 2 μmol/l), calcium currents and calcium-activated potassium conductances were abolished by intracellular perfusion of cells with KF. 2. Depolarizing voltage shifts from a holding potential of −90 mV yielded a fast transient outward current (I_K^f) and a delayed non-inactivating outward current (I_K^s). These currents could be separated by shifting the membrane potential to −50 mV, where I_K^f was almost completely inactivated. 3. DTX, at concentrations of 0.14–1.4 nmol/l selectively reduced a portion of the non-inactivating potassium current, leaving the transient outward current unaffected. Once manifested, the action of DTX could not be reversed by washing. 4. The I-V characteristic of the current blocked by DTX is almost linear and quite different from the one of the 'DTX-resistant' portion of I_K^s, which shows a non-linear I-V curve. 5. Tetraethylammonium (TEA, 30 mmol/l) strongly reduced I_K^f and I_K^s. However, subsequent application of DTX was still able to further reduce I_K^s. 6. 3,4-diaminopyridine (3,4-DAP, 500 μmol/l) unselectively reduced I_K^f and a portion of I_K^s. The remainder of the latter could not further be reduced by DTX, suggesting a similar action of the two blockers on non-inactivating potassium currents. 7. From the results presented, it is suggested that dendrotoxin selectively blocks a non-inactivating subtype of potassium channel.

Key words: Dendrotoxin – Potassium channel – Tetraethylammonium – 3,4-Diaminopyridine – Dorsal root ganglion

Introduction

Excitable membranes display a variety of potassium channels, differing in their physiological properties. However, only few substances are available which may serve to distinguish between the several types of channels pharmacologically. Tetraethylammonium (TEA) and aminopyridines are known to have distinct pharmacological effects (for review see Rogawski 1985). TEA is usually more effective in blocking non-inactivating potassium currents, whereas aminopyridines seem to preferentially affect inactivating, transient potassium currents. However, the 'specificities' are not always clear-cut, especially when high concentrations of these drugs are employed. It is therefore impossible to use either of the substances to completely eliminate a specific type of potassium channel without affecting another one. A novel scorpion toxin has been attributed to reduce potassium currents in squid axons (Carbone et al. 1982). However, it is not known whether this toxin acts on a specific type of potassium channel. Apamin, a component of the bee venom, has been reported to selectively reduce a certain type of Ca-dependent potassium current (Romey et al. 1984; Pennefather et al. 1985). But there is now evidence that apamin also inhibits calcium currents in heart muscle cells (Bkaily et al. 1985).

Dendrotoxin (DTX), a polypeptide isolated from the venom of the Green Mamba snake *Dendroaspis angusticeps* (Harvey and Karlsson 1980) has been reported to facilitate transmitter release from neuromuscular junctions (Harvey and Karlsson 1980, 1982; Harvey and Gage 1981). DTX also has been found to selectively block a fast transient outward potassium current in hippocampal slices from guinea-pig while leaving other ionic currents unaffected (Dolly et al. 1984). This effect may be responsible for convulsive states induced by DTX in the same preparation. Only recently Weller et al. (1985) presented evidence for the blockade of potassium currents by DTX in frog nerve fibres. However, no attempt was made to see whether the toxin exerts its effects specifically on non-inactivating or inactivating potassium currents which both are present in frog nerve fibres (Schwarz and Vogel 1971).

We have studied in detail the action of dendrotoxin on outward currents of guinea-pig dorsal root ganglion neurones, where at least two distinct voltage-activated potassium currents are present (Kostyuk et al. 1981b). Our findings strongly suggest that DTX, unlike in hippocampal cells, selectively blocks a portion of the persistent potassium current while leaving the fast transient outward current unaffected.

Methods

Preparation and intracellular dialysis. Experiments were performed on isolated neurones from dorsal root ganglia (DRG) of guinea-pigs (1–8 days old). The DRG were pretreated at 37°C for 20–50 min with trypsin (0.5%) and collagenase (type III, 0.1%) in a Ca^{2+}-Mg^{2+}-free solution containing (mmol/l): NaCl 138, KCl 2.6 and HEPES 10, adjusted to a pH of 7.4. After 30 min of washing with Earle's medium ($T = 20–23$°C) the DRG were mechanically disrupted to obtain single cells.

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Intracellular dialysis was essentially performed according to the technique described by Kostyuk et al. (1981a). Single cells ($\varnothing = 30-50$ µm) were sucked into a pore (8–15 µm) at the Sylgard-coated tip of a V-shaped plastic tube which was continuously perfused with the intracellular solution, containing (mmol/l): KF 140 and HEPES 10, buffered at pH 7.3. Intracellular access was gained when the membrane patch facing the pore ruptured due to the negative hydrostatic pressure. The tip of the tube was then inserted through a hole into another plastic tube which allowed to perfuse the extracellular part of the cells with a minimum of fluid. The extracellular solution contained (mmol/l): NaCl 136, KCl 2.6, $CaCl_2$ 1.8, $Mg\ Cl_2$ 0.5, HEPES 10, adjusted to a pH of 7.4.

Electrical measurements and separation of currents. The experimental set-up for voltage clamping was similar to that described in detail by Kostyuk et al. (1981a). Two Ag-AgCl electrodes accessing the intracellular solution served to measure the voltage and to pass current, respectively. Another Ag-AgCl wire in the bath served as the reference electrode (virtual ground). Transmembrane currents were recorded following stepwise shifts of the membrane voltage (duration: 500 ms) from holding potentials of -90 mV and -50 mV. Pulses had increments of 20 mV and were applied at intervals of 10 s. Signals were recorded, stored and processed on a digital oscilloscope (Nicolet 4094, Madison, USA). Cells were used which had membrane potentials between -45 to -65 mV. Their input resistances were in the range of 60–100 MΩ. Holding currents as well as the actual membrane potentials of cells were monitored to judge on the 'quality' of cells. Cells in which intolerable shifts of these parameters ocurred were discarded. All experiments were performed at room temperature (20–23° C).

To avoid the contribution of Na^+ inward currents only those results are presented which were obtained from neurones whose sodium currents could be abolished by tetrodotoxin (TTX, 2 µmol/l), although cells with TTX-resistant sodium currents (Kostyuk et al. 1981a) responded in a similar way to the various drugs tested. Ca^{2+} inward currents were abolished by the presence of intracellular fluoride ions (Kostyuk et al. 1981b). Consequently, Ca-activated potassium currents were not present. Leakage currents (less than 0.5 nA for a 40 mV shift) were assumed to be linear and compensated by an analogue circuit subtracting a voltage-proportional current step. This procedure seemed appropriate since no substantial effects of dendrotoxin on leakage currents were observed. Dendrotoxin was purified from *Dendroaspis angusticeps* venom (Harvey and Karlsson 1980; Weller et al. 1985) and found homogeneous in disc gel electrophoresis.

Results

Isolated neurones from guinea pig dorsal root ganglia (DRG) displayed at least two distinct potassium outward currents when subjected to depolarizing voltage shifts from a holding potential of -90 mV (Fig. 1A). The 'fast' current (which we will refer to as I_K^f) and the 'slow' current (I_K^s) could be separated when shifting the holding potential to -50 mV. At this potential I_K^f was almost completely inactivated, thus voltage steps from this level yielded the classical non-inactivating, delayed outward current I_K^s (Fig. 1D). The fast current (Fig. 1G) was obtained by subtraction of current traces from Fig. 1D from the corresponding current traces of Fig. 1A. It should be noted that the current obtained by this procedure showed a current portion which did not inactivate within 500 ms. This current is due to differences between the magnitudes of I_K^s elicited from the two holding potentials and may result from a long-term inactivation of the relevant channels when holding the cell at a voltage of -50 mV. Using the experimental approach described above we have investigated the effect of dendrotoxin (DTX) on outward currents and have compared it

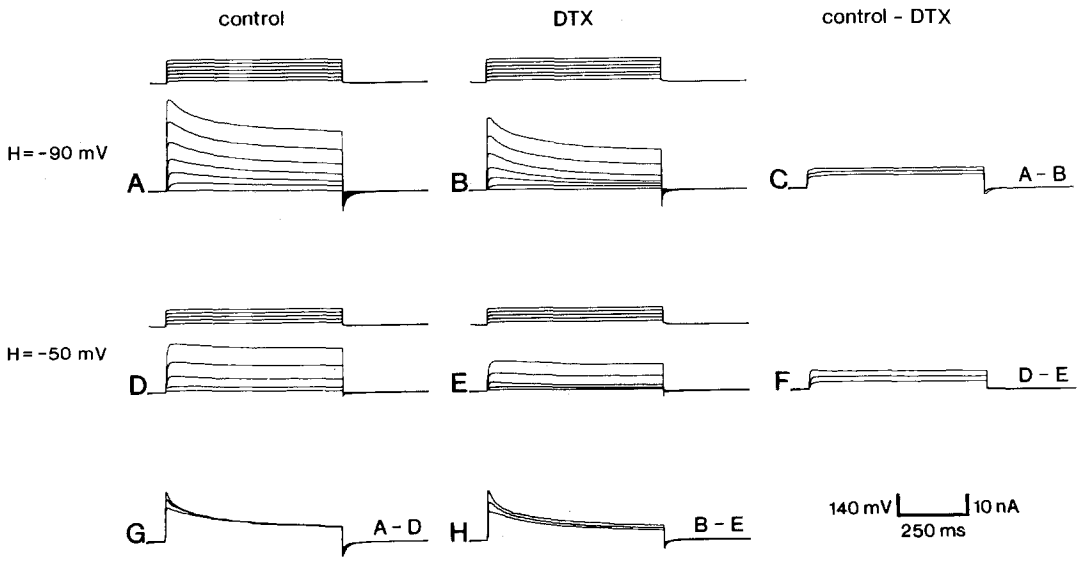

Fig. 1A–H. Effect of dendrotoxin (DTX) on outward potassium currents. **A, D** Outward currents (*lower traces*) elicited by depolarizing voltage steps (*upper traces*) from a holding potential of H = -90 mV and H = -50 mV, respectively. **B, E** Outward currents 10 min after application of DTX (1.4 nmol/l). **C, F** Currents blocked by DTX at H = -90 mV and H = -50 mV respectively. Traces were obtained by digital subtraction of corresponding current traces (**A–B**) and (**D–E**) at depolarizing voltage shifts to + 10/+ 30/+ 50 mV. **G, H** The fast current before and after application of DTX, respectively. Traces were obtained by subtraction of corresponding traces (**A–D**) and (**B–E**) at depolarizing voltage shifts to + 10/+ 30/+ 50 mV

with the actions of known potassium channel blockers like tetraethylammonium (TEA) and 3,4-diaminopyridine (3,4-DAP).

The action of externally applied DTX (1.4 nmol/l) on outward currents is demonstrated in Fig. 1B at a holding voltage of −90 mV and Fig. 1E at a holding voltage of −50 mV. When these currents are subtracted from the corresponding traces of the controls, the current which is blocked by DTX is obtained (Fig. 1C and 1F). The amount of current blocked by DTX was almost identical at the two holding potentials. It thus appears that only a portion of I_K^S is inhibited by the toxin. The selectivity of its action becomes more obvious when comparing the fast current after the application of DTX (Fig. 1H) with the control (Fig. 1G). No substantial effect on the peak current can be observed. However, a slight reduction of the non-inactivating component, which is also present in these traces is visible.

The onset of action of DTX was in the range of 2–10 min and dependent on the concentration employed. Low concentrations of DTX (0.14 nmol/l) required a longer time (5–10 min) for the onset of action and displayed a smaller reduction of potassium current than 1.4 nmol/l of the toxin. At this concentration the onset of action was in the range of 2–4 min and the reduction attainable was completed within 5–10 min. A typical example of these actions is shown in the I-V curves of Fig. 2A. The maximal reduction of the non-inactivating potassium current attainable with DTX (1.4 nmol/l) was 47% ± 11 (mean value ± SD, $n = 5$) for a depolarizing voltage shift to + 50 mV from a holding potential of − 50 mV. DTX at concentrations of 14 nmol/l or higher (up to 1.4 µmol/l) did not further reduce outward currents significantly (4 experiments). Once the action of DTX was completed the effects on potassium currents could not be reversed within times of 20–30 min of continuous washing. When DRG neurones were internally perfused with DTX (14 nmol/l) no reduction of outward currents could be observed.

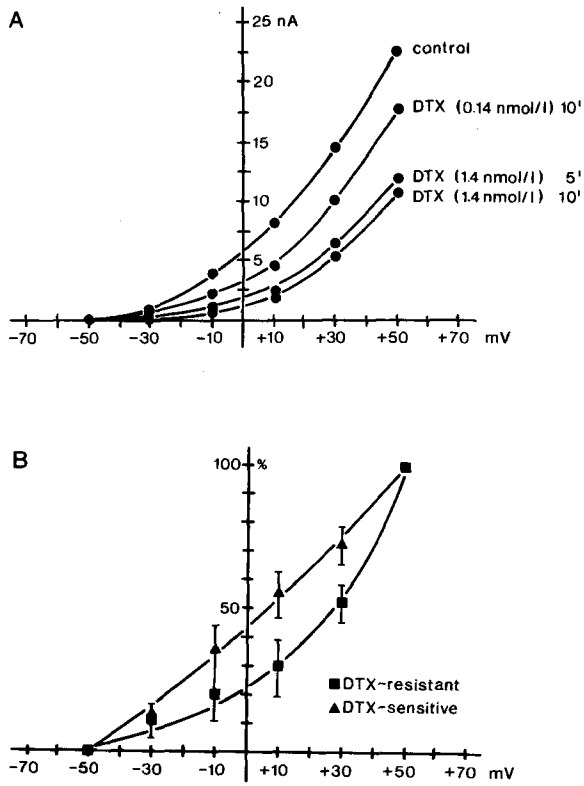

Fig. 2A,B. Effect of dendrotoxin (DTX) on the current-voltage relationship of the non-inactivating potassium current. Current amplitude were measured at the end of depolarizing voltage steps lasting 500 ms from a holding voltage of − 50 mV. **A** I-V relationship of outward potassium currents before and after the application of DTX as labelled in the graph. Data were acquired from the same as in Fig. 1. **B** I-V relationships of currents blocked by DTX (1.4 nmol/l, labelled DTX-sensitive) and currents which remained after application of the toxin (labelled DTX-resistant). For better comparison the currents were normalized by setting the current amplitude at + 50 mV to 100%. Values are means of five different cells (± SE). Curves were drawn by eye

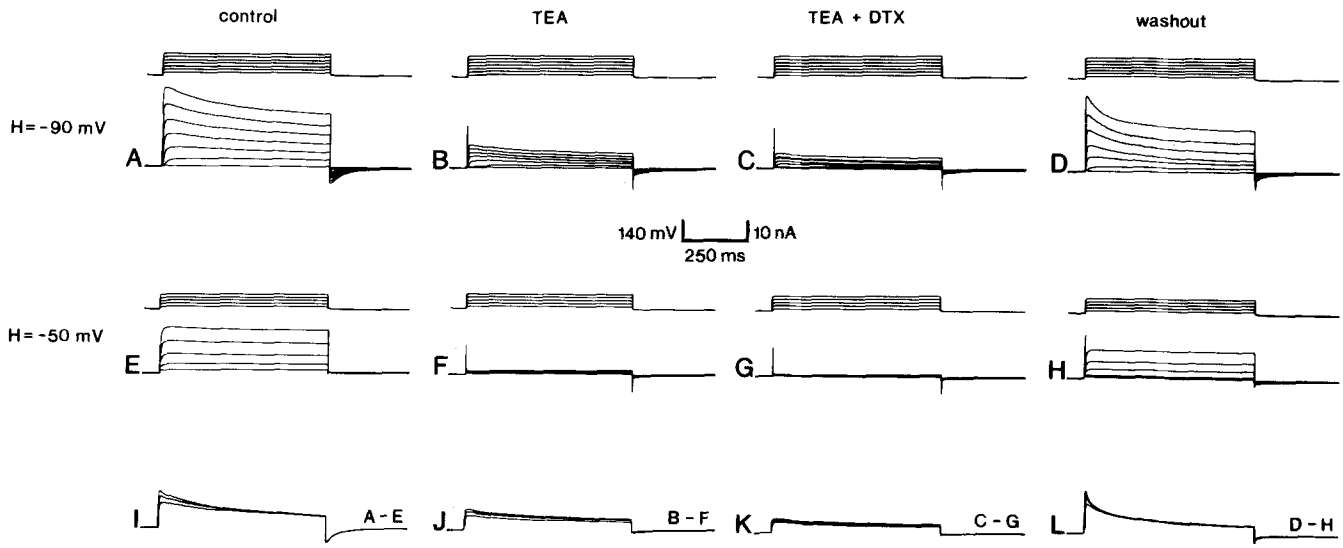

Fig. 3A–L. Effect of tetraethylammonium (TEA) and dendrotoxin (DTX) on outward potassium currents. **A, E** Outward currents (*lower traces*) elicited by depolarizing voltage steps (*upper traces*) from holding potentials of H = − 90 mV and H = − 50 mV, respectively. **B, F** Outward currents after extracellular application of TEA (30 mmol/l). **C, G** Currents 10 min after the additional application of DTX (1.4 nmol/l). **D, H** outward currents after 10 min of continuous washing. **I–L** The influence of TEA and DTX on the fast current. Traces were obtained by subtraction of corresponding traces (**A–E, B–F, C–G, D–H**) for voltage steps to + 10/+ 30/+ 50 mV

Interestingly, the degree of current reduction by DTX varied with respect to membrane potentials, prompting the question if the current blocked by DTX (1.4 nmol/l) displays a different I-V characteristic from the one of the current resistant to the toxin. Figure 2B illustrates the I-V relationships of the DTX-sensitive and the DTX-insensitive portion of I_K^s. For better comparison of the voltage dependence the currents were normalized by setting the current amplitude of the + 50 mV voltage shifts to 100%. From these data it appears that the I-V characteristic of the current blocked by DTX is quite different from the one of the control (e.g. Fig. 2A) or the current insensitive to DTX. Its I-V curve is almost linear suggesting the current to be carried by a distinct set of potassium channels. It should be noted that DTX had no significant effects on currents elicited by hyperpolarizing pulses.

For better evaluation of the action of DTX on potassium outward currents, we have compared it with the effects of other well-known potassium channel blockers. When externally applied, tetraethylammonium (TEA, 30 mmol/l) effectively reduced potassium outward currents elicited by depolarizing pulses from holding potentials of either − 90 mV (Fig. 3B) or − 50 mV (Fig. 3F). It thus appears that TEA nonspecifically blocks both fast and slow components of potassium outward currents. This is further demonstrated in Fig. 3J, where the effect of TEA on I_K^f is shown. When DTX (1.4 nmol/l) was applied in addition to TEA (Fig. 3C and G) outward currents were further reduced suggesting that DTX was still able to block potassium channels spared by TEA (5 experiments). After the washout of TEA and DTX (10 min) the signals obtained by depolarizing pulses from a holding voltage of − 90 mV and − 50 mV remained reduced (Fig. 3D and H, respectively). The current which remained blocked after the washout corresponded to the current which was usually abolished by DTX. The peak of I_K^f which normally was not affected by DTX yielded full recovery (Fig. 3L). The I-V relationships of I_K^s for this experiment are shown in Fig. 4. These results further suggest that DTX specifically acts on a portion of potassium channels constituing I_K^s in contrast to the action of TEA which nonspecifically reduced all outward currents. The effect of DTX was rather irreversible whereas the effect of TEA could easily be reversed by washing.

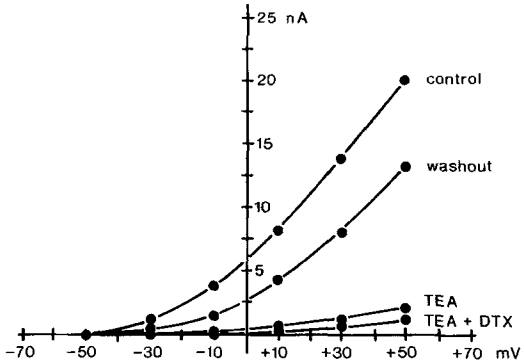

Fig. 4. Effect of tetraethylammonium (TEA) and dendrotoxin (DTX) on the current-voltage relationship of the non-inactivating potassium current. Data were acquired from the same cell as in Fig. 3. I−V curves, corresponding to the various treatments are labelled in the graph

3,4-Diaminopyridine (3,4-DAP), another potassium channel blocking agent, exerted a qualitatively similar action on outward currents compared to TEA. When externally applied, 3,4-DAP (500 μmol/l) reduced outward currents elicited by depolarizing pulses from a holding potential or either − 90 mV or − 50 mV. These actions are illustrated in Fig. 5B and E, respectively, the latter showing the time-dependent recovery of I_K^s during the depolarizing pulses as has been reported for squid axons (Kirsch and Narahashi 1978). It thus appears that 3,4-DAP at this high concentration not only inhibits I_K^f (Fig. 5H) but also a portion of I_K^s. The amount of non-inactivating current blocked by 3,4-DAP was similar to the portion of I_K^s usually affected by DTX. When DTX was applied in addition to 3,4-DAP

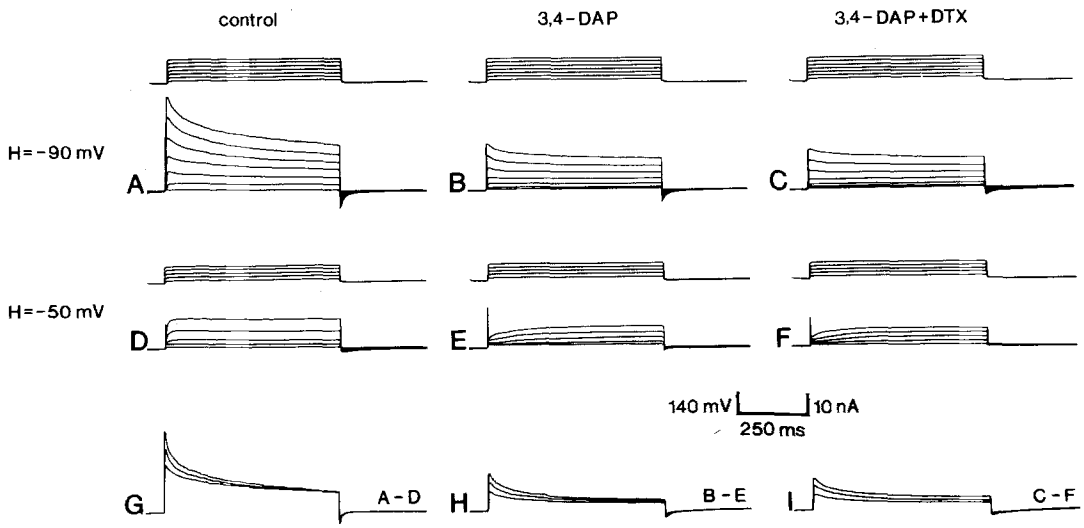

Fig. 5A−I. Effect of 3,4-diaminopyridine (3,4-DAP) and dendrotoxin (DTX) on outward potassium currents. **A, D** Outward currents (*lower traces*) elicited by depolarizing voltage steps (*upper traces*) from holding potentials of H = − 90 mV and H = − 50 mV, respectively. **B, E** Outward currents after extracellular application of 3,4-DAP (500 μmol/l). **C, F** Currents 10 min after the additional application of DTX (1.4 nmol/l). **G−I** The influence of 3,4-DAP and DTX on the fast current. Traces were obtained by subtraction of corresponding traces (**A−D, B−E, C−F**) for voltage steps to + 10/+ 30/+ 50 mV

(4 experiments), no further reduction of potassium currents was observed (Fig. 5C, F and I), suggesting the current blocked by 3,4-DAP to be identical with the current blocked by DTX. It should be noted that after washing for 10–15 min only a slight recovery of I_K^f could be obtained and that I_K^s remained blocked. In two other experiments where DTX (1.4 nmol/l) had already reduced I_K^s, 3,4-DAP (500 µmol/l) only inhibited I_K^f, leaving the remaining I_K^s unaffected (not illustrated).

Discussion

While dendrotoxin (DTX), was found to selectively block the fast transient outward potassium current (I_A) of voltage clamped hippocampal cells (Dolly et al. 1984), our experiments with DTX on dorsal root ganglion (DRG) neurones revealed a selective reduction of a portion of the non-inactivating potassium current (I_K^s) while leaving the inactivating outward current I_K^f unaffected. DTX exerted its effects at concentrations as low as 0.14–1.4 nmol/l. The reduction of potassium current was similar in all cells investigated. It was in the range of 35–65% and no further reduction could be obtained by concentrations as high as 1.4 µmol/l. Once manifested, the depressing effects of DTX on the persistent potassium current could not be reversed within 20–30 min of continuous washing. Irreversibility of action was also observed in the hippocampal cells (Dolly et al. 1984), whereas in the frog ranvier node the action of DTX could partially be reversed by washing for 26 min (Weller et al. 1985).

The I-V characteristic of the current blocked by DTX is almost linear and quite different from that of the control current and the portion of I_K^s which remains unaffected by DTX (Fig. 2B). From this finding it is suggested that the non-inactivating potassium current found in DRG neurones may be carried by two distinct sets of potassium channels, one of which is DTX-resistant and shows a voltage dependence, whereas the other one is DTX-sensitive and displays a rather ohmic behaviour. Another possible explanation for this finding, which cannot be ruled out by our experiments, may be an effect of DTX on the voltage sensitivity of potassium channels carrying I_K^s.

Tetraethylammonium (TEA) at a concentration of 30 mmol/l effectively reduced outward currents. I_K^f as well as I_K^s were strongly reduced, suggesting nonspecific blockade of all types of potassium currents activated, including the ones sensitive to DTX. Subsequent application of DTX further reduced the already small remainder of I_K^s, indicating the presence of potassium channels spared by TEA. After washing for several minutes full recovery of the fast current was obtained, whereas I_K^s remained reduced. The current which stayed blocked corresponds to the current which is typically inhibited by DTX. These findings further indicate that DTX selectively blocks a portion of the non-inactivating potassium current. The incomplete recovery of I_K^s is not likely to be due to a run-down of potassium currents, since I_K^f which is usually more susceptible to run-down phenomena showed full recovery.

From biochemical data (Weller et al. 1985) and from the actions of DTX on the transient potassium outward current in hippocampal sclices (Dolly et al. 1984) it has been suggested that DTX has an aminopyridine-like action. Our experiments with 3,4-diaminopyridine (3,4-DAP) show that the action of 3,4-DAP at high concentrations (500 µmol/l) is not restricted to a reduction of I_K^f but also a portion of I_K^s is inhibited. Subsequent application of DTX did not further reduce I_K^s significantly. Likewise, a reduction of I_K^s by 3,4-DAP could not be observed after DTX had reduced the persistent potassium current, whereas I_K^f, which is not affected by DTX was reduced. These findings confirm the aminopyridine-like action of DTX with respect to I_K^s. However, DTX seems to be specific for I_K^s, whereas 3,4-DAP additionally blocks the fast current.

From the results presented it is suggested that DTX reduces outward current confined to a subtype of non-inactivating potassium channels. The action of DTX is highly selective and rather irreversible. The toxin may therefore be used as a pharmacological tool to separate potassium currents. It may also serve as a marker of a certain type of potassium channel.

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