

Oxaliplatin, an Anticancer Agent that Affects both Na⁺ and K⁺ Channels in Frog Peripheral Myelinated Axons

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Abstract. The use of oxaliplatin, a relatively new chemotherapeutic agent, is somewhat limited since it produces a specific peripheral neuropathy regarding other neurotoxic anticancer platinum analogues. In order to investigate the mechanism of such a peripheral neuropathy, the effects of 1–100 $\mu\text{mol/l}$ oxaliplatin were assessed on the nodal ionic currents of single frog myelinated axons as a model of peripheral excitable membranes. Oxaliplatin decreased both Na⁺ and K⁺ currents in a dose-dependent manner and within 5–10 min, without producing any marked changes in the current kinetics. It was about three to eight times more effective in reducing the Na⁺ than the K⁺ current. In addition, it shifted the voltage-dependence of both Na⁺ and K⁺ conductances towards negative membrane potentials. A negative shift in the steady-state inactivation-voltage curve of the peak Na⁺ current was also observed in the presence of oxaliplatin. These effects were not reversed by washing the myelinated axons with an oxaliplatin-free solution for at least 30 min. It is concluded that oxaliplatin modifies the voltage-dependent ionic channels mainly by altering the external surface membrane potential. The knowledge of such a mechanism may help to counteract the neurotoxic action of this anticancer agent.

Key words: Oxaliplatin — Anticancer agent — Peripheral myelinated axons — Potassium and sodium channels — Voltage-clamp technique

Introduction

Oxaliplatin, a relatively new platinum compound with a 1,2-diaminocyclohexane carrier ligand (Fig. 1), is registered in Europe and almost all over the world (including USA and Japan) for the first line treatment of advanced colorectal cancer (Grothey and Goldberg 2004), and is under evaluation in several good clinical practice studies on other tumour types (Lorusso 2000; Misset et al. 2000; Zaniboni and

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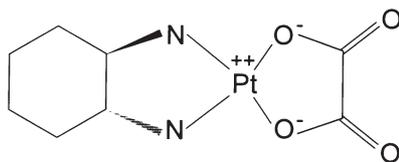


Figure 1. Chemical structure of oxaliplatin.

Meriggi 2005). This anticancer agent has a demonstrated broad spectrum of activity both *in vitro* and *in vivo*. Its mechanism of action is similar to that of other chemotherapeutic analogues such as cisplatin and carboplatin, but the *in vitro* screening shows different spectra of activity and toxicity, probably related to the characteristics of the compound. Indeed, oxaliplatin is of interest because, unlike other platinum derivatives, it is not associated with significant nephrotoxicity or ototoxicity. Its dose-limiting toxicity is an unusual form of peripheral neuropathy (Extra et al. 1998; Cassidy and Misset 2002; Grothey 2005).

Hence, oxaliplatin induces a specific acute neurotoxicity characterised by rapid onset (frequently during infusion or a few hours after), high prevalence (i.e. 90% of patients involved), short lasting symptoms (some hours to a few days) in correlation with the infusion rate and consisting in mild to moderate dysesthesias and paresthesias that can be worsened or triggered by cold and are sometimes associated with cramps, tetanic spasms and myotonia. This cluster of symptoms is aggravating and heavily disturbing for the patients. In addition, oxaliplatin was reported to induce a cumulative neurotoxicity, similar to that produced by cisplatin and characterised by moderate to severe peripheral neuropathy (that can temporally impair functions requiring fine co-ordination, the symptoms being described as regressive within a few months after treatment discontinuation), as well as by paresthesias (that persist between chemotherapy cycles, i.e. 2–3 weeks). This cumulative neurotoxicity has a low incidence, i.e. between 10 to 15% of patients treated with cumulative doses of at least 680 mg/m² (Extra et al. 1998; Cassidy and Misset 2002; Grothey 2005).

While the oxaliplatin-induced cumulative neurotoxicity could be explained, if not completely at least partially, by the accumulation of platinum adducts in the dorsal root ganglion (see McKeage et al. 2001; Kweekel et al. 2005), emerging data are trying to elucidate the mechanisms of oxaliplatin-induced acute neurotoxicity. Among the different theories, one of the most convincing involves alterations of voltage-dependent Na⁺ channels as reported by studies performed on the Na⁺ current recorded from rat dorsal root ganglia and cockroach dorsal unpaired median neurons (Adelsberger et al. 2000; Grolleau et al. 2001). However, the mechanisms proposed by these recent studies to explain the oxaliplatin-induced acute neurotoxicity, are somewhat controversial.

In this context, the aim of the present work was to analyse the effects of oxaliplatin on voltage-dependent ionic channels of the peripheral nervous system

of amphibian, widely used as a model of peripheral excitable membranes to study the mechanism of action of various agents. For this purpose, the effects of 1–100 $\mu\text{mol/l}$ oxaliplatin were investigated on the nodal membrane Na⁺ and K⁺ currents of single frog myelinated axons, using a conventional voltage-clamp technique.

Materials and Methods

Preparation and technique

The experiments were performed on nodes of Ranvier of single sensory or motor myelinated axons isolated from the sciatic nerve removed from adult male frogs (*Rana esculenta*) weighing 20–25 g. All efforts were made to minimise the suffering of frogs (i.e. they were rapidly decapitated and demedullated), and a minimal number of animals was used. All animal experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Single nerve fibres of about 0.5 cm length were isolated and mounted in a five-compartment chamber as designed and described elsewhere (Stämpfli and Hille 1976). The nodal membrane currents were recorded under voltage-clamp conditions using the method of Nonner (1969). Between pulses, the nodal membrane was maintained at the normal resting potential of fibres, assumed to be -70 mV and corresponding to 30% inactivation of peak Na⁺ current. The node of Ranvier under investigation was stimulated at a frequency of 0.5–1 Hz. Membrane currents were calculated assuming an axoplasmic resistance of 10 M Ω . Linear leakage and capacitative currents were subtracted electronically from the total current. The series resistance was not compensated (for details, see Benoit et al. 1985). Experiments were performed at 15–16 °C.

After amplification, electrical signals were displayed on a digital oscilloscope Tektronix 2224 (Les Ulis, France) and simultaneously recorded on video cassette tape Biologic DTR-1200 (Claix, France). Data were collected and analysed on a microcomputer equipped with an analogue/digital interface board, model DT2821 (Data Translation Inc, Marlboro, USA) using the Acquis1 software kindly provided by Gérard Sadoc (CNRS, Gif sur Yvette, France). The sampling frequency was 40 kHz.

To describe the activation state of K⁺ and Na⁺ conductances, the experimental values of conductance g were calculated using the following equation:

$$g = I/(V - V_{\text{eq}}) \quad (1)$$

where I is the current amplitude, V is the test voltage and V_{eq} is the ionic equilibrium potential.

Statistical analysis of data was performed using the Student's t -test (two-tailed). Data were expressed as the mean value \pm standard error of the mean (SEM) of n myelinated axons, and were considered significant at $p < 0.05$.

Solutions and chemicals

The node of Ranvier under investigation was superfused with a standard physiological solution or with test solutions. The standard physiological solution had the following composition (in mmol/l): 111.5 NaCl; 2.5 KCl; 1.8 CaCl₂; 10 HEPES (N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid) buffered at pH 7.4 with NaOH. The nerve fibre ends were cut in a solution containing 120 mmol/l KCl, which was used in the end pools of the recording chamber throughout the experiments.

In some experiments, when monitoring K⁺ current, the Na⁺ current was suppressed by adding 1 μmol/l tetrodotoxin, purchased from Sigma (Saint Quentin Fallavier, France), to the external solution. In some other experiments, when recording Na⁺ current, the K⁺ current was suppressed by replacing the end pool solution with 110 mmol/l CsCl + 10 mmol/l NaCl, and by adding 10 mmol/l tetraethylammonium hydrochloride, purchased from Sigma (Saint Quentin Fallavier, France), to the external solution.

Oxaliplatin (*trans*-1-diaminocyclohexane oxaliplatinum, molecular weight: 397.47) was obtained from Sanofi-Synthelabo (Eloxatin[®], Sanofi-Synthelabo, France). It was first prepared as a 10 mmol/l solution in water and then diluted with the standard physiological solution, prior to use, to give final concentrations in the range of 1 to 100 μmol/l.

Results

Effects of oxaliplatin on the nodal Na⁺ current

The effects of oxaliplatin were first studied on the Na⁺ current associated with long-lasting (45 ms) depolarizing voltage steps to 0 mV (Fig. 2A). As shown in this typical experiment, oxaliplatin (10 and 100 μmol/l) consistently decreased the amplitude of the Na⁺ current, when applied to the solution bathing single myelinated axons. This effect was dependent on the concentration of oxaliplatin used. Indeed, after about 5 to 7 min, the peak Na⁺ current was decreased by 10 and 100 μmol/l of agent to respectively 77 ± 8% (*n* = 11) and 41 ± 7% (*n* = 10) of its control value (see also Fig. 6), and then remained statically unchanged during 10–15 min. It should be noted that no additional effect of oxaliplatin was detected on the Na⁺ current. In particular, no significant change in the current kinetics was observed during the action of the agent as compared to control conditions (see inset in Fig. 2A).

An example of the reduction of the peak Na⁺ current induced by either 10 or 100 μmol/l oxaliplatin at various test voltages is presented in Fig. 2B. The reversal potential of the peak Na⁺ current was not affected by the agent. This indicates that oxaliplatin did not modify the ionic selectivity of Na⁺ channels. However, in addition to its blocking effect, the agent shifted the peak Na⁺ current-voltage curve towards negative membrane potentials. Therefore, the Na⁺ current activated at more negative potentials in the presence than in the absence of oxaliplatin. In

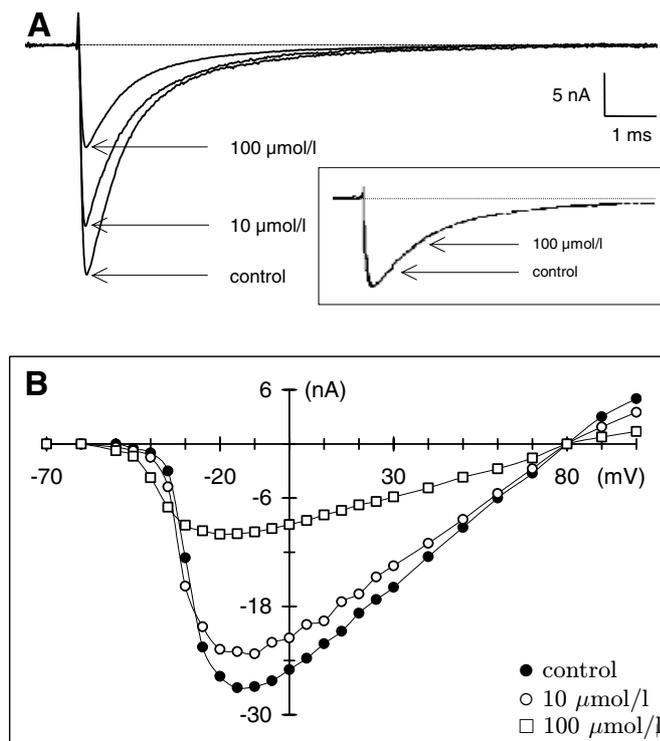


Figure 2. Effects of oxaliplatin on the nodal Na⁺ current. **A.** Traces of Na⁺ current recorded during depolarizing voltage steps to 0 mV, preceded by 50 ms hyperpolarizing voltage steps to -120 mV, before (control) and after the addition of either 10 or 100 μmol/l oxaliplatin to the external solution. In the inset, the current recorded in the presence of 100 μmol/l of agent was normalised and superimposed to that recorded under control conditions. The straight lines indicate the zero current level. **B.** Peak Na⁺ current-voltage relationships. The peak Na⁺ current was measured during depolarizations of various amplitudes, preceded by 50 ms periods of hyperpolarization to -120 mV, under control conditions (●) and in the presence of either 10 μmol/l (○) or 100 μmol/l (□) oxaliplatin.

particular, it was activated at -50 mV with 10 or 100 μmol/l of agent whereas it was zero under control conditions. The effects of oxaliplatin on the Na⁺ current were not reversed after 30 to 40 min washing of the myelinated axons with an agent-free solution (not shown).

Effects of oxaliplatin on the nodal K⁺ current

External applications of oxaliplatin (10 and 100 μmol/l) to myelinated axons also produced a dose-dependent decrease in the K⁺ current recorded during depolarizing voltage steps to 90 mV, without significantly modifying the current kinetics (Fig. 3A). After about 5 to 7 min of oxaliplatin exposure, the steady-state K⁺

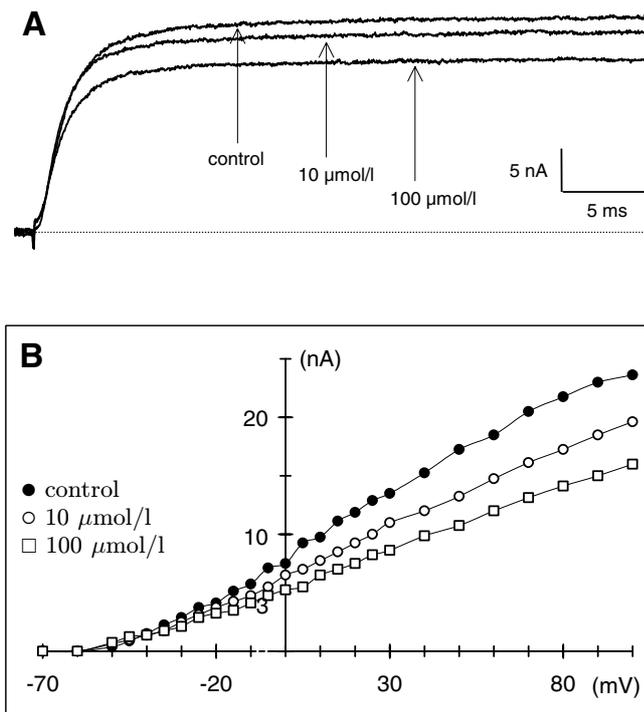


Figure 3. Effects of oxaliplatin on the nodal K⁺ current. **A.** Traces of K⁺ current recorded during 45 ms periods of depolarization to 90 mV, before (control) and after the addition of either 10 or 100 μmol/l oxaliplatin to the external solution. The straight line indicates the zero current level. **B.** Steady-state K⁺ current-voltage relationships. The K⁺ current was measured at the end of 45 ms depolarization periods of various amplitudes, under control conditions (●) and in the presence of either 10 μmol/l (○) or 100 μmol/l (□) oxaliplatin.

current was reduced to $92 \pm 5\%$ ($n = 5$) and $65 \pm 9\%$ ($n = 6$) of its control value in the presence of 10 and 100 μmol/l of agent, respectively (see also Fig. 6), and then remained statically unchanged during additional 10–15 min.

The blocking effect of oxaliplatin on the K⁺ current occurred at any membrane potential tested. This is shown by the steady-state K⁺ current-voltage relationships established before and after the addition of 10 and 100 μmol/l of agent to the external physiological solution (Fig. 3B). Here again, the effects of the agent on the K⁺ current were not reversed after washing the myelinated axons with an oxaliplatin-free solution for at least 30 min (not shown).

Effects of oxaliplatin on Na⁺ and K⁺ conductance-voltage relationships

The effects of oxaliplatin were analysed on the voltage-dependence of the Na⁺ and K⁺ current activation by studying the action of the agent on the peak Na⁺ and

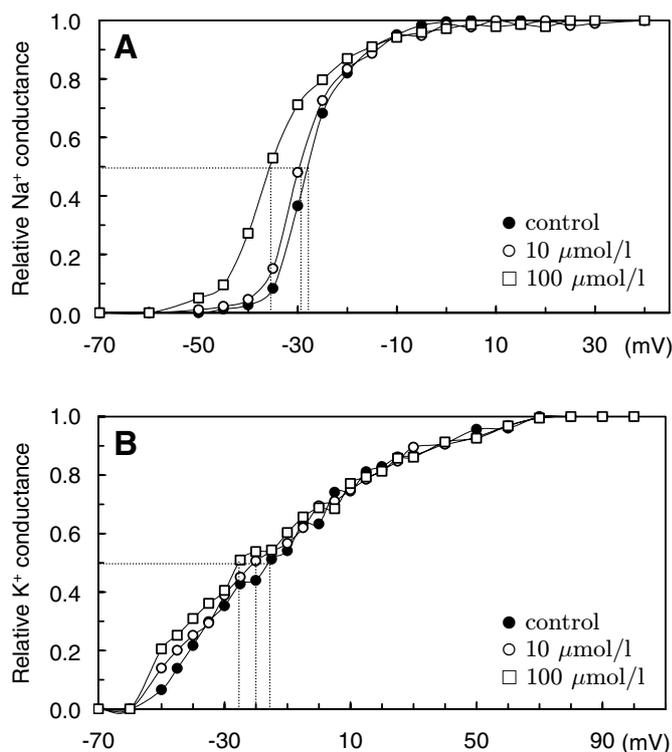


Figure 4. Peak Na⁺ (A) and steady-state K⁺ (B) conductance-voltage relationships, under control conditions (●) and in the presence of either 10 μmol/l (○) or 100 μmol/l (□) oxaliplatin. The peak Na⁺ current was measured during depolarizations of various amplitudes, preceded by 50 ms periods of hyperpolarization to -120 mV. The K⁺ current was measured at the end of 45 ms depolarization periods of various amplitudes. In each medium, the conductance was calculated as described in the text, normalised to its maximum value at large positive membrane potentials, and plotted against membrane potential during test pulses. The straight lines indicate the membrane potential corresponding to half-maximum conductance. The differences between the membrane potential corresponding to half-maximum conductance in the presence of 10 and 100 μmol/l oxaliplatin and under control conditions were -3 and -8.5 mV, respectively (A), and -5 and -9 mV (B).

steady-state K⁺ conductance-voltage curves (Fig. 4). For each test voltage, the peak Na⁺ and steady-state K⁺ conductances were calculated in the absence and in the presence of 10 or 100 μmol/l oxaliplatin according to Eq. 1 where the equilibrium potential of Na⁺ ions was the reversal potential of the peak Na⁺ current and that of K⁺ ions, calculated from the Nernst equation, was -96 mV under our experimental conditions (see Materials and Methods).

Fig. 4 shows that oxaliplatin shifted the peak Na⁺ and steady-state K⁺ con-

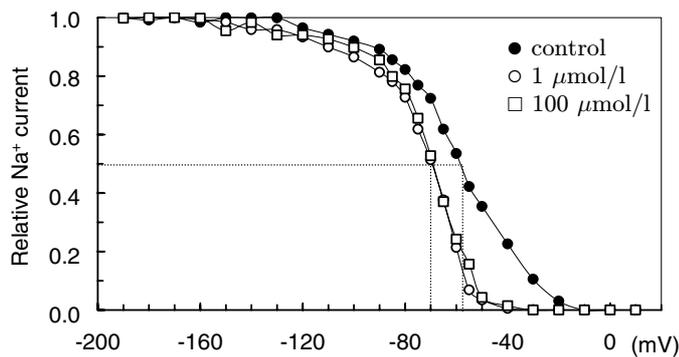


Figure 5. Steady-state Na^+ current inactivation-voltage relationships, under control conditions (\bullet) and in the presence of either $1 \mu\text{mol/l}$ (\circ) or $100 \mu\text{mol/l}$ (\square) oxaliplatin. In each medium, the peak Na^+ current was measured during depolarizations to 0 mV preceded by 50 ms pulses of various amplitudes, normalised to its maximum value at large negative membrane potentials, and plotted against membrane potential during pre-pulses. The straight lines indicate the membrane potential corresponding to half-maximum steady-state Na^+ current inactivation. The differences between the membrane potential corresponding to half-maximum steady-state Na^+ current inactivation in the presence of 1 and $100 \mu\text{mol/l}$ oxaliplatin and under control conditions were -12 and -11 mV , respectively.

ductance-voltage curves towards negative membrane potentials. The value of the negative shifts was calculated as the difference between the test voltage corresponding to half-maximum conductance in the presence of the agent and that under control conditions. It was dependent on oxaliplatin concentration and was $-3.5 \pm 0.5 \text{ mV}$ ($n = 4$) and $-8.8 \pm 0.3 \text{ mV}$ ($n = 3$) with respectively 10 and $100 \mu\text{mol/l}$ of agent for the peak Na^+ conductance-voltage curve, and $-3.8 \pm 1.2 \text{ mV}$ ($n = 3$) and $-9.5 \pm 0.5 \text{ mV}$ ($n = 3$) with respectively 10 and $100 \mu\text{mol/l}$ of agent for the steady-state K^+ conductance-voltage curve.

Effects of oxaliplatin on the steady-state Na^+ current inactivation-voltage curve

The voltage-dependence of the steady-state Na^+ current inactivation was studied using the classical two-pulse protocol, in the absence and in the presence of 1 and $100 \mu\text{mol/l}$ oxaliplatin (Fig. 5). The main effect of the agent was to shift the steady-state inactivation-voltage curve of the peak Na^+ current towards negative membrane potentials. The membrane potential corresponding to half maximum steady-state inactivation of the peak Na^+ current was not significantly dependent on the concentration of oxaliplatin used between 1 and $100 \mu\text{mol/l}$. It was $8.5 \pm 2.8 \text{ mV}$ ($n = 3$) and $10 \pm 2.2 \text{ mV}$ ($n = 3$) more negative in the presence of 1 and $100 \mu\text{mol/l}$ of agent, respectively, than under control conditions. As a consequence, oxaliplatin was more effective in decreasing the peak Na^+ current as the membrane potential during pre-pulses was increasingly less negative (see also Fig. 6).

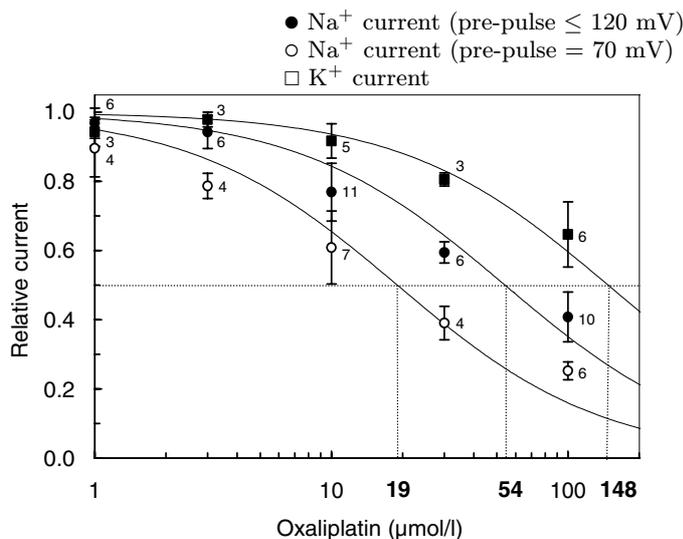


Figure 6. Dose-response relationships of the effects of oxaliplatin on Na⁺ and K⁺ currents. The peak Na⁺ current was measured during depolarizing voltage steps to 0 mV preceded (●) or not (○) by 50 ms hyperpolarizing voltage steps ≤ −120 mV, before and after the addition of 1–100 μmol/l oxaliplatin to the external solution. The steady-state K⁺ current (□) was measured at the end of 45–60 ms periods of depolarization to 90 mV, before and after the addition of 1–100 μmol/l oxaliplatin to the external solution. In each case, the currents were normalised to their respective values in the absence of oxaliplatin, and plotted against the agent concentration. The curves, drawn by regression analyses through the points ($r^2 > 0.98$, which means that the fits explain 98% of the total variation in the data about the average), represent the inhibition of currents assuming a one-to-one reaction between channels and oxaliplatin molecules. The agent concentrations corresponding to a 50% decrease in currents (straight lines) were 148 μmol/l (□), 54 μmol/l (●) and 19 μmol/l (○). Each point represents the mean value ± SEM of n myelinated axons (number beside the point).

Dose-response relationships of the effects of oxaliplatin on Na⁺ and K⁺ currents

Finally, the peak Na⁺ and the steady-state K⁺ currents were measured before and after treatment of myelinated axons with 1 to 100 μmol/l oxaliplatin, normalised to their respective values without agent, and expressed as a function of oxaliplatin concentration. The results are presented in Fig. 6. It is clear that the agent was more effective, on one hand, in decreasing the Na⁺ than the K⁺ current and, on the other hand, in decreasing the Na⁺ current when the membrane potential applied to axons during pre-pulses was less negative. Indeed, the concentration of oxaliplatin corresponding to a 50% decrease in Na⁺ current was about three times lower upon the pre-pulse potential being close to the resting membrane potential of myelinated axons (i.e. −70 mV, see Materials and Methods) instead to more negative membrane potentials (i.e. ≤ −120 mV), and was about

three to eight times lesser than that corresponding to a 50% decrease in the K^+ current.

Discussion

The present results reveal that external applications of 1–100 $\mu\text{mol/l}$ of the new antitumoural platinum analogue oxaliplatin to frog myelinated axons consistently produced a decrease in the Na^+ current and, in a lesser extent, in the K^+ current, without inducing any significant changes in the current kinetics. In addition, the agent shifted the voltage-dependence of Na^+ and K^+ current activation and that of Na^+ current inactivation towards negative membrane potentials.

Oxaliplatin-induced blockade of Na^+ and K^+ channels

The oxaliplatin concentration needed to produce a 50% decrease in Na^+ current was 54 $\mu\text{mol/l}$ (Fig. 6). A similar inhibition of the Na^+ current was previously reported in cockroach dorsal unpaired median neurons (Grolleau et al. 2001). However, in these neurons, the agent was primarily active when intracellularly applied and when the Na^+ current exhibited a sustained component rather than a complete inactivation. An oxaliplatin-induced slight inhibition of the Na^+ current was also reported in primary culture of rat dorsal root ganglia neurons treated with high concentrations of agent, while no apparent effect could be detected on the Na^+ current recorded from primary cultures of rat hippocampal neurons (Adelsberger et al. 2000). This strongly suggests that oxaliplatin discriminates between Na^+ channel isoforms, and acts specifically on certain of those isoforms that are expressed mainly in the peripheral nervous system. It is worth noting that oxaliplatin did not produce any marked slow down inactivation of the Na^+ current in frog myelinated axons (Fig. 2A) in contrast to what is pointed out for rat dorsal root ganglia neurons (Adelsberger et al. 2000). This indicates that neither state (resting, open or inactivated) preferential blockade of Na^+ channels nor modifications of their kinetic properties occurred during the action of the agent. Therefore, the present work shows that oxaliplatin is able to block Na^+ channels under conditions that closely match those clinically used (see Introduction and below).

Oxaliplatin produced not only a decrease in the Na^+ current but also a reduction of the K^+ current. The concentration of 148 $\mu\text{mol/l}$ of agent was needed to cause a 50% decrease in K^+ current (see Fig. 6). Oxaliplatin was thus about three times less effective in blocking K^+ than Na^+ channels in frog myelinated axons. It is the first time that the agent is shown to affect voltage-dependent ionic channels other than Na^+ channels. Indeed, from indirect experiments performed *in vitro* on electrotonic responses of the rat sural nerve, it was concluded that 250 $\mu\text{mol/l}$ of oxaliplatin had no effect on K^+ channels (Adelsberger et al. 2000). This may indicate that the effects of oxaliplatin are restricted to certain isoforms of not only Na^+ channels (see above) but also K^+ channels. Moreover, 500 $\mu\text{mol/l}$ of agent failed to block the high-voltage-activated Ca^{2+} current characterised in cockroach dorsal unpaired median neurons (Grolleau et al. 2001). It should be noted that the K^+

current kinetics were not significantly modified by oxaliplatin (Fig. 3). This indicates that, as mentioned above for Na⁺ channels, neither state preferential blockade of K⁺ channels nor modifications of their kinetic properties occurred during the action of the agent.

Oxaliplatin-induced negative shifts in the voltage-dependence of channel activation and inactivation

In addition to produce a decrease in both Na⁺ and K⁺ currents, oxaliplatin also shifted the voltage-dependence of their activation towards negative membrane potentials (Fig. 4). As a consequence, the percentage of activated Na⁺ and K⁺ channels was increased at a given membrane potential during the action of the agent. It should be emphasised that a similar negative shift in the voltage-response relationship was previously reported for the Na⁺ current recorded from rat dorsal root ganglia neurons treated with the agent (Adelsberger et al. 2000). Moreover, oxaliplatin also shifted the voltage-dependence of Na⁺ current inactivation towards negative membrane potentials (Fig. 5). As a consequence, at a given membrane potential, the percentage of inactivated Na⁺ channels was increased during the action of the agent. Therefore, oxaliplatin was about three times more effective in decreasing the Na⁺ current when the membrane potential during pre-pulses was increasingly less negative and, in particular, when it was close to the resting membrane potential of myelinated axons, i.e. -70 mV (see Materials and Methods), rather than to more negative values, i.e. ≤ -120 mV (see Fig. 6).

It is well known that cations, and in particular Ca²⁺ ions, unspecifically screen the fixed negative charges that are located at the outer surface of the membrane and are involved in the control of the external surface membrane potential (Hille 1968). Furthermore, in myelinated axons, evidence have been given for the existence of a high density of such fixed negative surface charges close to the gating sensors of the Na⁺ and K⁺ channel proteins (Hille 1968; Neumcke and Stämpfli 1984; Benoit 1993; Elinder and Arhem 1998). Therefore, the oxaliplatin-induced hyperpolarizing shifts in the voltage-dependence of Na⁺ and K⁺ channel gating parameters may result from modifications of the transmembrane potential due to alterations of cations that screen the negative surface charges. Indeed, these results can be perfectly explained in terms of screening effects if one assumes that the agent directly or indirectly produces a decrease in the external concentration of Ca²⁺ ions. In agreement with this proposal, oxaliplatin was previously reported to exert its effects *via* a chelation of Ca²⁺ ions through the action of its metabolite oxalate (Grolleau et al. 2001). It should be noted that, in addition, some interactions may exist between oxaliplatin molecules and axonal membrane elements, since this agent was shown to block Na⁺ and K⁺ channels (see above). Moreover, it is likely that such interactions are irreversible if one considers that the effects of oxaliplatin were not reversed under our experimental conditions (see also Adelsberger et al. 2000). Interestingly, the value of the oxaliplatin-induced negative shifts in the voltage-dependence of Na⁺ current inactivation (Fig. 5), in contrast to Na⁺ and K⁺ current activation (Fig. 4), was not significantly dependent on the concentra-

tion of agent used between 1 and 100 $\mu\text{mol/l}$. This would strongly suggest that the fixed negative surface charges are not uniformly distributed near the Na^+ and K^+ channel gates (see also Neumcke and Stämpfli 1984; Benoit 1993). Moreover, these results also indicate that 1 $\mu\text{mol/l}$ oxaliplatin was a concentration high enough to produce maximum effects on the steady-state Na^+ current inactivation-voltage relationship.

Relevance of the effects of oxaliplatin with its clinical neuropathy

The clinical dose of oxaliplatin usually administrated to patients (3.61 mg *per l*, i.e. about 10 $\mu\text{mol/l}$; Graham et al. 2000) is in the range of the concentrations of agent used in the present study (i.e. between 1 to 100 $\mu\text{mol/l}$). As stated above, such oxaliplatin concentrations consistently modified the voltage-dependence of Na^+ and K^+ channel gating parameters by altering the external surface membrane potential and, in a lesser extent, blocked Na^+ and K^+ channels.

In amphibians, as in human and other mammals, the activation of Na^+ channels is well known to be responsible for the depolarization of the nodal membrane that occurs during the propagation of action potentials through myelinated axons, whereas the inactivation of Na^+ channels is involved in the membrane repolarization (Frankenhaeuser and Huxley 1964; Chiu and Ritchie 1981a; Schwarz et al. 1995). Therefore, the oxaliplatin-induced negative shifts in the voltage-dependence of Na^+ channel activation will result in an increased membrane excitability since, in the presence of the agent, the action potentials will be generated at more negative membrane potentials than they normally are. Moreover, the oxaliplatin-induced negative shifts in the voltage-dependence of Na^+ channel inactivation will contribute to increase the membrane excitability by decreasing the duration of action potentials. Similarly, the oxaliplatin-induced negative shifts in the voltage-dependence of K^+ channel activation are likely to also contribute to increase the membrane excitability by decreasing the duration of action potentials since the activation of K^+ channels is also involved in the membrane repolarization that occurs during the propagation of action potentials through amphibian and, under certain conditions, human and mammalian myelinated axons (Frankenhaeuser and Huxley 1964; Chiu and Ritchie 1981b; Schwarz et al. 1995). It is worth noting that the percentage of Na^+ channels blocked by oxaliplatin concentrations close to the therapeutic dose is not enough important to lead to marked effects of the agent on the amplitude of propagated action potentials (Frankenhaeuser and Huxley 1964).

In addition to a specific and high prevalent acute neurotoxicity, oxaliplatin was reported to induce a cumulative neurotoxicity, similar to that produced by cisplatin although having a much lower incidence (see Introduction). It was postulated that the acute neurotoxic symptoms induced by oxaliplatin might be caused by cellular mechanisms different from those of the cumulative neurotoxicity observed with both drugs (Adelsberger et al. 2000). Indeed, it was recently shown that the intracellular accumulation of cisplatin is highly dependant on the functioning of Na^+, K^+ -ATPase $\alpha 1$ subunit (Kishimoto et al. 2006). Although some more experiments are needed to definitively conclude, one can assume that ox-

oxaliplatin may influence the three main systems that are dominant in sodium and potassium homeostasis, i.e. Na⁺ channels, K⁺ channels and Na⁺,K⁺-ATPases.

Conclusion

It is suggested that the clinical neuropathy of oxaliplatin, characterised by a membrane hyperexcitability, results from modifications of the voltage-dependent ionic channels mainly due to alterations of the external surface membrane potential. Although further experiments are needed to be definitely concluded, it is likely that cations, and in particular Ca²⁺ ions, that unspecifically screen the fixed negative charges located at the outer surface of the membrane, are involved in the effects of the agent. The knowledge of such a mechanism, if transferred to the clinical practice, may help to counteract the neurotoxic effects of oxaliplatin, which would provide a real benefit to the patients in terms of quality of life and tolerability. Indeed, the agent-induced neurotoxicity was reported to be highly reduced when Ca²⁺ and Mg²⁺ ions were infused to patients before and after oxaliplatin administration (Gamelin et al. 2004).

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Final version accepted: May 2, 2006