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European Journal of Pharmacology 406 (2000) 25–32

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The chemotherapeutic oxaliplatin alters voltage-gated Na^+ channel kinetics on rat sensory neurons

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Received 19 June 2000; received in revised form 22 August 2000; accepted 24 August 2000

Abstract

The chemotherapeutic oxaliplatin causes a sensory-motor neuropathy with predominantly hyperpathic symptoms. The mechanism underlying this hyperexcitability was investigated using rat sensory nerve preparations, dorsal root ganglia and hippocampal neurons. Oxaliplatin resulted in an increase of the amplitude and duration of compound action potentials. It lengthened the refractory period of peripheral nerves suggesting an interaction with voltage-gated Na^+ channels. Application of oxaliplatin to dorsal root ganglion neurons resulted in an increase of the Na^+ current, a block of the maximal amplitude and a shift of the voltage–response relationship towards more negative membrane potentials. The effect was detectable on 13 of 18 tested cells. This observation, together with the absence of any effect on Na^+ currents of hippocampal neurons, suggests that the interaction of oxaliplatin is restricted to one or more channel subtypes. The effect of oxaliplatin could be antagonised by the Na^+ channel blocker carbamazepine which could be used to reduce side effects of oxaliplatin therapy in patients.

Keywords: Neuropathy; Colorectal cancer; Dorsal root ganglia; Hippocampal neuron; Sural nerve

1. Introduction

Oxaliplatin (*trans*-l-diaminocyclohexane oxaliplatinum) is a platinum-based chemotherapeutic agent with a 1,2-diaminocyclohexane carrier ligand. Its antitumoural activity has been proven in clinical trials (Becouarn and Rougier, 1998; Gerard et al., 1998). Oxaliplatin differs from other routinely used platinum-based cytostatics like cisplatin in its lack of nephrotoxicity and from carboplatin in its low hematologic toxicity. There is no cross-resistance to cisplatin (Extra et al., 1998; Raymond et al., 1998). Phase I and II studies indicate that peripheral neuropathy is the most severe side-effect of oxaliplatin therapy. The neurotoxic profile of oxaliplatin is particular in its rapid onset, location and intensity of sensory disturbance in the absence of a motor component (Extra et al., 1998). The peripheral sensory neuropathy is characterised by dysaesthesia and/or distal paraesthesia (fingers, toes and, less frequently, peri-oral region and pharyngo-laryngeal tract), induced or exacerbated by cold. When treatment is continued, the extent of the symptoms can increase and their duration can be prolonged to the extent of becoming permanent, causing functional impairment. This is the most frequent dose-limiting toxicity of oxaliplatin (Gerard et al., 1998). The incidence and intensity of neurologic side effects according to World Health Organization (WHO) specific scale grade 1–3 is dose-related (Extra et al., 1990; Gerard et al., 1998).

Clinical electrophysiological investigations were done in several studies. The findings were in favour of moderate sensory-motor axonal degeneration and myelin loss (Extra et al., 1990; Levi et al., 1993). These minor lesions are very different from the axonal degeneration observed in vincristine- or cisplatin-induced neuropathies which become predominantly severe in large myelinated fibres. Mammalian sensory nerves consist of myelinated, fast

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0014-2999/00/$ - see front matter
PII: S0014-2999(00)00667-1

conducting A-fibres and unmyelinated, slow conducting C-fibres. The latter mainly transmit nociception and pain, whereas A-fibres play a role in sensing temperature and other sensory modes. The intensity of the subjective signs observed in patients, particularly dysaesthesia to cold, contrasts with the minor morphological abnormalities found mainly in small unmyelinated fibres. Little is known about the acute effects of oxaliplatin on nerve excitability on the cellular level.

It is thought that the oxalate group of the molecule is displaced in a high chloride environment such as blood by Cl^- resulting in 1,2-diaminocyclohexane-platinum-dichloride. After entering the intracellular space where the Cl^- concentration is low the Cl^- ions are released and the compound interconverts to the mono- and diaquated-subtypes (Raymond et al., 1998) which are thought to be the active molecules. Thus, no enzymatic reaction seems to be involved in this transformation. It is unclear how oxaliplatin enters the cells. Apart from passive diffusion through membranes, more specific pathways via transporters may also be involved. The mechanism underlying the neurological side effects of oxaliplatin is unknown so far. In a former study, it was shown that oxaliplatin depressed Na^+ and K^+ currents in myelinated frog nerves (Boughattas, 1994) and that it enhanced the amplitudes and durations of compound action potentials on rat sural nerve preparations (Adelsberger et al., 1999). In the present study, we demonstrate actions of oxaliplatin on compound action potentials and electrotonic potentials of rat sural, peroneal and vagal nerve preparations and on Na^+ currents of rat dorsal root ganglia and hippocampal neurons.

2. Materials and methods

2.1. Preparations and solutions

Seven sural, three peroneal and three vagal nerves from adult rats were prepared to lengths of about 3–4 cm and stored in a modified Krebs' solution containing (in mM): NaCl (118.1), KCl (3.4), $MgSO_4$ (0.8), KH_2PO_4 (1.2), $NaHCO_3$ (25.0), $CaCl_2$ (2.5) bubbled with 95% O_2–5% CO_2, pH 7.4 at room temperature. After stripping off the perineural sheath the nerves were transferred into the recording chamber. Tetrodotoxin, 4-aminopyridine, tetraethylammonium and carbamazepine were purchased from Sigma (Deisenhofen, Germany). Oxaliplatin (Eloxatin, Sanofi-Synthelabo) was obtained from the international pharmacy or Sanofi-Synthelabo and predissolved to a concentration of 12.5 mM with H_2O_{bidest} to avoid precipitation. This stock solution was prepared daily and stored under light protection.

Recordings of Na^+ currents from single cells were performed using primary cultures of hippocampal neurons from neonatal rats and dorsal root ganglia neurons from adult rats. Dorsal root ganglia were cut in smaller pieces and incubated in phosphate-buffered saline containing 0.125% (w/v) collagenase type V (Sigma) at 37°C for 1 h. Dissociation of the tissue to single cells was then achieved by passage through a series of injection needles (20 G down to 26 G). After each passage, the supernatant containing single cells was collected. After centrifugation, cells were seeded on glass coverslips and incubated in F12 medium containing 10% (v/v) fetal calf serum at 37°C for 2–3 days. Round cells with diameters between 20 and 40 μm were used for the recording of Na^+ currents.

Hippocampal neurons were plated on poly-D-lysine coated glass coverslips after mechanical dissociation. The neurons were cultured 7–14 days together with their astrocytes in minimum essential medium (MEM, GIBCO), supplemented with transferrin (1 mg/ml), insulin (5 mg/ml), progesteron (20 nM), putrescin (100 mM), selendioxide (30 nM), sodium-pyruvate (1 mM) and ovalbumin (0.1%).

2.2. Experimental set-up

Compound action potentials and electrotonic potentials were recorded at 37°C in a three-chambered Marsh ganglion bath (Hugo Sachs Elektronik, Hugstetten, Germany) (Marsh et al., 1987; Grafe et al., 1994). Each partition had removable lower and upper sections with a small slot to allow the nerve to pass between the compartments without being crushed. Silicone grease was used to seal the nerve in position and to avoid free diffusion of solutions between the compartments. The middle compartment had a volume of 1.5 ml and was continuously perfused at a flow rate of 10 ml/min. The K^+ concentration in the right compartment was elevated to 30 mM. The nerve ending in the left lateral compartment was drawn into a suction electrode which was used for application of voltage pulses to elicit compound action potentials and current pulses for recording of electrotonus. For compound action potentials, the nerve was stimulated in the left compartment while for electrotonic measurements, stimulation was applied along the left and the central compartment. The supramaximal stimulus for action potentials was 100 V with durations between 15 μs and 2 ms, and electrotonic potentials were recorded after application of current steps of up to ±15 μA and durations of 18 or 180 ms. Recordings of potential differences (DC potential) across the resistance between the central and the right compartment were performed using a pair of DC-stable Ag/AgCl recording electrodes. Stimulation, pulse protocol and registration were performed using pClamp software (Axon Instruments, USA) on a personal computer.

For patch clamp recordings of voltage-gated Na^+ currents, dorsal root ganglia neurons were superfused with a solution containing (in mM): NaCl (162), KCl (5.3), HEPES (15), $CaCl_2$ (2), Glucose (5.6) pH 7.4 adjusted with NaOH, and hippocampal neurons with a solution containing (in mM): NaCl (140), KCl (5), $MgCl_2$ (1),

$CaCl_2$ (2), HEPES (10), $CoCl_2$ (2) pH 7.3 adjusted with NaOH. Patch pipettes were pulled from borosilicate glass with an outer diameter of 1.5 mm to a tip resistance of 2–2.5 MΩ with a DMZ-puller (Zeitz Instruments, Munich, Germany). For recordings from dorsal root ganglia neurons, pipettes were filled with a solution containing (in mM): CsCl (140), $MgCl_2$ (2), EGTA (11), HEPES (10), pH 7.4 adjusted with CsOH and for recording from hippocampal neurons with a solution containing (in mM): CsF (120), CsCl (20), 1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (10), HEPES (10), ATP-Na_2 (2) pH 7.2 adjusted with CsOH. Whole-cell recordings were carried out in the voltage-clamp mode (Hamill et al., 1981) using a EPC9 (HEKA, Lamprecht, Germany) patch clamp amplifier and filtered with 1 kHz. Cells were voltage-clamped to −80 mV (dorsal root ganglia neurons) and −60 mV (hippocampal neurons), and Na^+ currents were evoked by depolarizing voltage steps of 20–200 ms starting from −80 to −60 mV, respectively, in increments of 10 mV at a frequency of 1 Hz. Drugs were applied by bath application. All patch clamp recordings were performed at room temperature.

3. Results

3.1. Oxaliplatin increased compound action potentials in A-fibres

Compound action potentials of rat sural and vagal nerves in normal physiological solution showed a clear distinction between A- and C-fibres, depending on the stimulus strength and duration, as previously described by Lambert and Dyck (1993) for human sural nerves. Fig. 1A (control) shows a typical action potential elicited by a stimulus of 100 V and 15 μs length, generated by the A-fibre component of the nerve. The action potential of these fast, myelinated axons started immediately after the stimulus and rose within 500 μs to a maximal value of about 0.6 mV. Then it declined again with a similar time course to the initial level. About 5–10 min after application of 250 μM oxaliplatin to the bath the time course and size of the action potential changed. After a 45-min exposure to oxaliplatin, the size of the potential had almost doubled (increase in A-fibre compound action potential amplitude of all nerves investigated: 218 ± 68%; $n = 7$). The time course of the decline increased dramatically, and after 45 min the baseline was reached after 10 ms, in contrast to 500 μs in the control measurement. Interestingly, the rise time of the action potential was not affected by oxaliplatin, well demonstrated in the superimposed presentation of the recorded traces (Fig. 1A). This effect of oxaliplatin could not be reversed even at prolonged washout times of more than 60 min. Therefore, a new nerve preparation had to be used for each application of oxaliplatin.

The activation of the C-fibre component of the sural nerve longer stimuli were necessary. Due to the slower

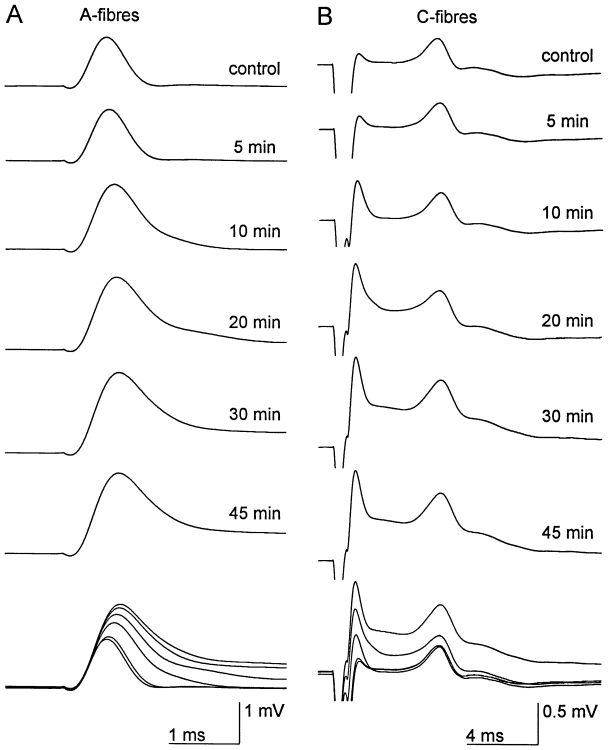

Fig. 1. Compound action potentials of rat sural nerve elicited in control solution and 5, 10, 20, 30 and 45 min after application of 250 μM oxaliplatin to the bath. The lower panel shows the single traces superimposed for better comparison of the changes caused by oxaliplatin. Each trace is an average of three recordings elicited at a frequency of 0.1 Hz. (A) A-fibre component of the compound action potential elicited by a supramaximal 15 μs stimulus at 100 V. (B) Subsequent stimulation with a 2-ms stimulus to additionally elicit C-fibres. Under these conditions the A-fibre component is partially masked by the long stimulus artefact.

conductance of unmyelinated C-fibres, the respective action potential appeared with a delay to the stimulus of about 4–8 ms. In the experiment shown (Fig. 1B), the action potential was elicited by a pulse of 100 V and 2 ms duration. Therefore, the A-fibre response was hidden in part by the longer stimulus artefact. The size of the action potentials of C-fibres was in the range of 0.05 mV, and they showed longer onset and decay time courses than the action potentials of A-fibres. Surprisingly, only little effect of oxaliplatin on the C-fibre response could be detected in all seven experiments of this type using the same concentrations and conditions (increase in C-fibre compound action potential amplitude: 36 ± 29%; $n = 7$). The apparent baseline shift of traces in the superimposed presentation was caused by the influence of oxaliplatin in this mixed A- and C-fibres preparation on the A-fibre component of the action potential which made it difficult to evaluate the size, as well as the time course, of the C-fibre component alone. During the compound action potential registrations, the DC potential was continuously monitored to detect shifts (in DC) reflecting a possible hyper- or depolarizating-effect of the drug. However, no significant shift in DC potential was observed ($n = 3$, data not shown).

3.2. Oxaliplatin caused repetitive firing in electrotonic responses

Electrotonic responses of sural nerve preparations to symmetric 18 and 180 ms current pulses are shown in Fig. 2. Electrotonic potentials were induced by depolarizing currents in normal bathing solution (control) and after application of 250 μM oxaliplatin ($n = 5$). Responses started with a fast Na^+ current rising within less than 1 ms and declining with a similar time constant due to the deactivation of the Na^+ channels and the opposing effect of the K^+ current arising with a small delay. After about 5 ms the firing had stopped under control conditions. Several minutes after addition of oxaliplatin, repetitive firing started and was clearly detectable after 15 min, especially at high time resolution (Fig. 2A). This effect kept increasing, and after 60 min it was clearly visible throughout the 180 ms pulses. The time of firing was lengthened from about 5 ms in control solution to several hundred ms after 60 min of exposure to oxaliplatin. This effect increased further throughout the time of application, and no saturation could be detected even after 2.5 h. As already observed for the compound action potentials, this effect could not be reversed by washout but kept increasing during the washout procedure.

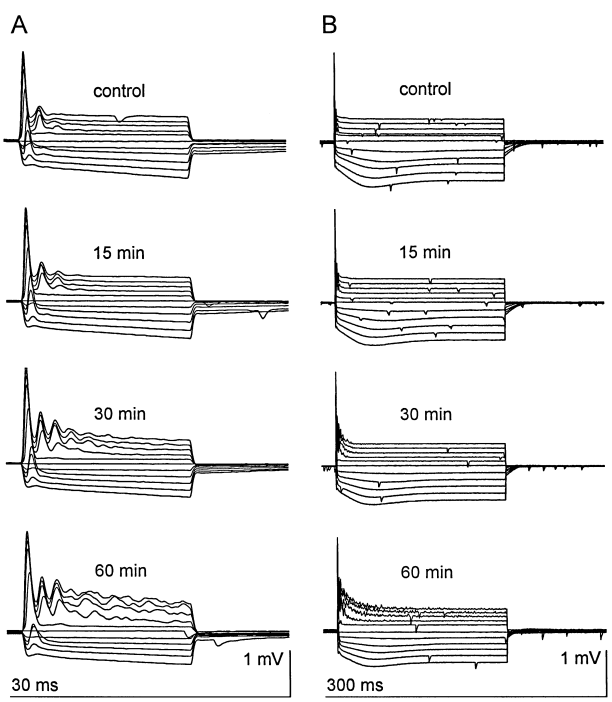

Fig. 2. Electrotonic responses of a rat sural nerve preparation elicited by 18 (A) and 180 ms (B) de- and hyper-polarizing current pulses applied to the same preparation in normal bathing solution and 15, 30 and 60 min after addition of 250 μM oxaliplatin to the bath. Each trace of the 11 recordings is an average of two recordings with 15 μA current steps which were performed for each recording series and are presented in superimposition. Upward deflection is due to depolarization and downward deflection is due to hyperpolarization.

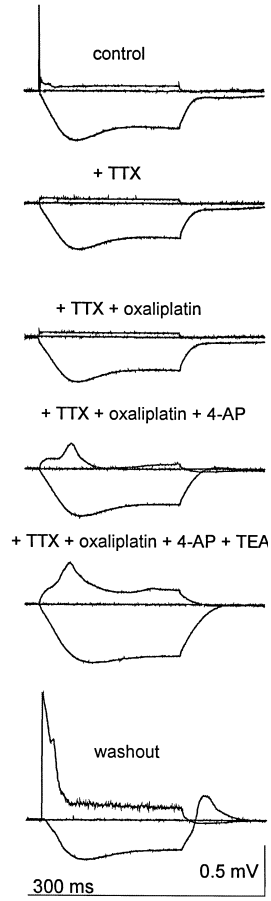

Fig. 3. Electrotonic responses of a rat sural nerve preparation under control conditions and after application of 1 μM TTX, 1 μM TTX + 250 μM oxaliplatin (30 min incubation), 1 μM TTX + 250 μM oxaliplatin + 3 mM 4-AP/3 mM TEA (15 min incubation) and after 30 min washout. Only the response to one hyper-and one depolarizing pulse is shown. The length of the current pulses was 180 ms. TTX = tetrodotoxin. 4-AP = 4-aminopyridine. TEA = tetraethylammonium.

3.3. Oxaliplatin did not act as a K^+ channel blocker

In the next step of the study, possible actions of oxaliplatin on K^+ channels were investigated. Electrotonic responses to 180 ms current pulses were recorded under control conditions (Fig. 3). Addition of 1 μM tetrodotoxin to the bath resulted in a complete block of the Na^+ current within 2 min. The remaining electrotonus consisted of a fast component which appeared instantaneously after the current pulse and was almost symmetrical in the depolarizing and the hyperpolarizing direction. This component represents the passive cable response and is mainly determined by the nodal capacitance and resistance and the capacitance and resistance of the myelin sheath (Bostock et al., 1983). The slower components of the electrotonus represent rectifying K^+ channels which also depolarize the nerve and which can be blocked by 4-aminopyridine and tetraethylammonium (Baker et al., 1987).

Addition of 250 μM oxaliplatin to a nerve pretreated with tetrodotoxin (Fig. 3 + TTX) showed no influence on

the electrotonic response after 30 min of incubation (Fig. 3 + TTX + oxaliplatin). Ten minutes after addition of 3 mM 4-aminopyridine and 3 mM tetraethylammonium, a block of fast and slow K^+ channels could be detected. Effective washout of tetrodotoxin, 4-aminopyridine and tetraethylammonium from this preparation could be performed within 5–10 min. Therefore, after washout of the blockers, only the irreversible effect of oxaliplatin on the electrotonus remained. The incubation time with oxaliplatin in the presented experiment was 90 min, demonstrating again a continued increase of the effect during incubation when compared to Fig. 2A, where the maximal incubation time was 60 min.

3.4. Oxaliplatin lengthened the refractory period of the nerve

After exclusion of a block of K^+ channels, an influence of oxaliplatin on Na^+ channels seemed most likely. Therefore, the refractory period was tested, which is primarily determined by the inactivation kinetics of Na^+ channels. In Fig. 4 (control), A-fibre action potentials were elicited by twin pulse stimulation of a sural nerve. The twin pulse interval started with 30 ms and then declined to 2 ms in increments of 2 ms. Under control conditions, the amplitude of the second action potential reached the amplitude of the first one at intervals of 10 or more ms. Addition of 250 μM oxaliplatin resulted in an increase of both the amplitude and duration of all action potentials. The twin pulse interval had to be increased to 30 ms ($n = 2$) to achieve similar amplitudes of the two action potentials (Fig. 4).

3.5. The effect of oxaliplatin could be antagonised by carbamazepine

Peroneal and vagal nerves are characterized by a higher content of C-fibres compared to the sural nerve. Therefore,

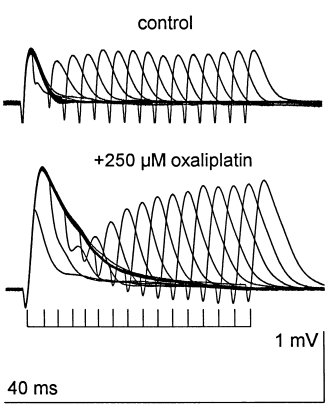

Fig. 4. Twin pulse stimulation of a sural nerve under control conditions and after 90 min incubation with 250 μM oxaliplatin. The twin pulse interval was varied by increments of 2 ms. Twin pulse frequency 0.1 Hz. A-fibre action potentials were elicited by supramaximal stimuli of 15 μA at 100 V.

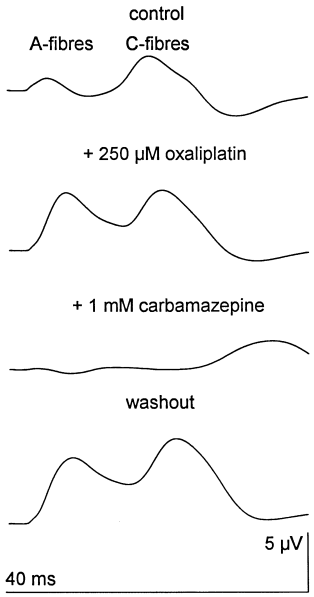

Fig. 5. Compound action potential of a vagal nerve elicited by stimuli of 15 μA at 100 V with a frequency of 0.1 Hz showing the small A-fibre component and a larger C-fibre component under control conditions, 45 min after addition of 250 μM oxaliplatin, after addition of 1 mM carbamazepine and after washout of carbamazepine demonstrating the remaining oxaliplatin effect. Note the increase in latency of the C-fibre component under carbamazepine.

the C-fibre component of the compound action potential of this nerves are larger as visible in recordings performed with a vagal nerve preparation (Fig. 5). Application of 250 μM oxaliplatin resulted in an increase of the A-fibre response as shown in Fig. 1. Again, the effect of the drug was more prominent on A-fibre than on C-fibre compound action potentials, increasing their amplitude twofold without altering the latency. Additional application of 1 mM carbamazepine, an antiepileptic drug known to act as a Na^+ channel blocker (Willow et al., 1984), abolished the A-fibre compound action potential and considerably reduced the C-fibre compound action potential which was accompanied by an increase in latency within a few minutes ($n = 2$). After washout of carbamazepine, the Na^+ channel block reversed and the compound action potential could be detected again with the enhanced A- and C-fibre component caused by oxaliplatin.

3.6. The effect of oxaliplatin was restricted to Na^+ channels on peripheral neurons

To specify the effect of oxaliplatin, Na^+ currents were recorded from dorsal root ganglia and hippocampal neurons. The Na^+ currents on dorsal root ganglia neurons were typically activated at potentials of -30 to -50 mV with maximal amplitudes in the range of -10 to -40 mV. After the rapid onset of the current within a few milliseconds to the maximal amplitude, inactivation occurred with one to several time constants depending on

each individual cell (Fig. 6A). 250 μM oxaliplatin influenced the Na^+ current in 13 of 18 cells tested. The effect was characterised by a shift of the voltage–response relationship of 10 mV towards more negative potentials and by a reduction of the maximal amplitude to 0.65 ± 0.23 ($n = 13$). The most pronounced effect was a slowdown of the inactivation kinetics of the channels. The degree of this effect varied between cells with an average increase of the total current by a factor of 2.6 ± 1.45 ($n = 13$), measured as the area under the current trace. The action of oxaliplatin on single cells started within 1 min after bath application and was, in contrast to the effects on whole nerve preparations, fully reversible after washout times of 2–5 min.

Na^+ currents elicited in hippocampal neurons showed more than one peak, especially at more negative membrane potentials, caused by different subtypes of Na^+ channels on the cells (Fig. 6B). In contrast to dorsal root ganglia neurons, no effect of oxaliplatin on Na^+ currents on hippocampal neurons could be detected ($n = 8$).

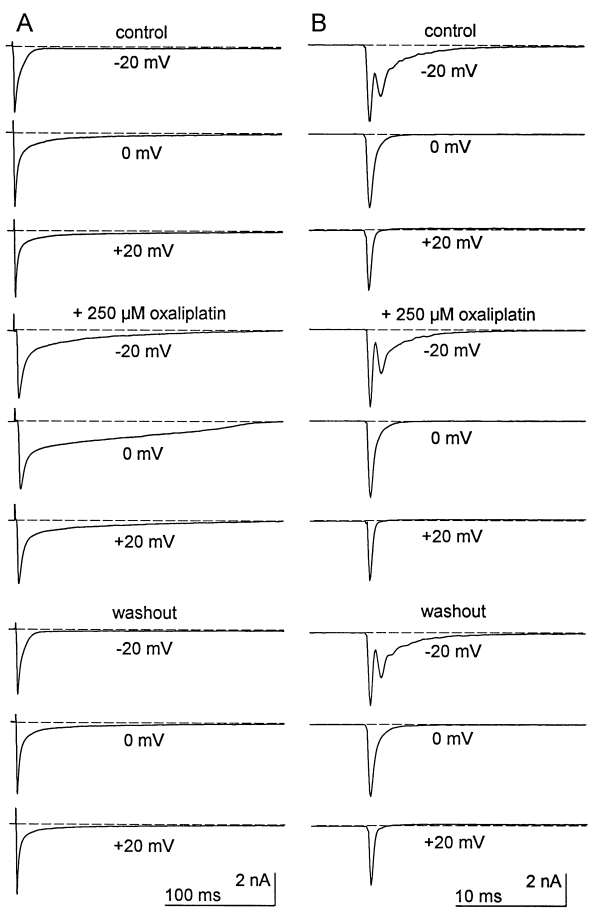

Fig. 6. Sodium currents of dorsal root ganglia neurons (A) and hippocampal neurons (B). Cells were clamped to −80 mV (dorsal root ganglia neurons) and −60 mV (hippocampal neurons) and currents were elicited by depolarising steps by an increment of 10 mV. Stimulus lengths were 300 ms for dorsal root ganglia and 50 ms for hippocampal neurons. Traces show currents elicited by voltage steps to −20, 0 and +20 mV under control conditions, after 5 min application of 250 μM oxaliplatin and after 5 min washout.

4. Discussion

Oxaliplatin belongs to a family of platinum-derived chemotherapeutic drugs, which are known to cause predominantly sensory neuropathy. The peculiar characteristic of oxaliplatin is its ability to induce acute dysaesthesia and hyperalgesia right after administration which cannot be explained by morphological changes of the nerve. Therefore, oxaliplatin must have a direct effect on nerve excitability which has not been known of the other platinum compounds. Based on results from myelinated frog nerves, it was suggested that these neurotoxic effects of oxaliplatin may be caused by a block of Na^+ and K^+ currents (Boughattas, 1994). In the present study, we have been able to demonstrate that oxaliplatin does not block K^+ conductances but alters the activation and inactivation behaviour of Na^+ channels. We used sural, vagal and peroneal nerves from the rat, because a mammalian preparation is more closely related to human nerves. Moreover, the acute neurological disorders reported by patients under treatment with oxaliplatin are mainly sensory without a major motor component. We investigated effects of oxaliplatin on compound action potentials and electrotonic responses at various concentrations of the drug ranging from 25 to 250 μM. At all concentrations, the observed effects were the same, except for a later onset after application of lower concentrations. Isolated axonal preparations will gradually die after a few hours in vitro, even without any treatment. By then, one would see a mixture of the oxaliplatin effect and effects caused by alterations of the preparation. To avoid incubation times of more than 3 h and to restrict the time of observation to the average life span of an intact preparation, most of the experiments were performed at 250 μM oxaliplatin. Even here, effects were delayed by at least 5–10 min, but kept increasing during further incubation with the drug and showed no saturation. Most probably, this is due to a prolonged diffusion time of the substance through the surrounding connective tissue and/or the glial sheath. This hypothesis is also based on the observation that the effect of oxaliplatin was irreversible on whole nerve preparations and reversible in the single cell preparation.

After an action potential is elicited by a depolarizing pulse, the fast depolarizing current is carried by an ion influx through Na^+ channels. Termination of the action potential occurs through inactivation of the Na^+ channels and the subsequent repolarization of the axon by an efflux of K^+ through K^+ channels. Therefore, an increase both in size and in duration of the action potential, as observed under oxaliplatin (Figs. 1A and 5), could be due either to a block of K^+ channels or to a slowdown of Na^+ channel

inactivation kinetics or both. In electrotonic responses, both effects would be visible as enhanced and persistent repetitive firing, as shown in Fig. 2. To test for a possible block of K^+ channels by oxaliplatin, we recorded electrotonic responses under block of the Na^+ current by tetrodotoxin (Fig. 3). After application of oxaliplatin the remaining K^+ current was not changed and could still be blocked by incubation with the K^+ channel blockers 4-aminopyridine and tetraethylammonium. After washout of all effectors, a large and persistent effect of oxaliplatin on electrotonic responses was detectable. These experiments demonstrate that oxaliplatin could not mimic the effects caused by K^+ channel blockers, favouring the suggestion that the chemotherapeutic has an influence on Na^+ channel kinetics.

A slowdown of Na^+ channel inactivation kinetics should result in an increase of the refractory time of the nerve. Refractory times were determined by twin pulse stimulation with various pulse intervals, under control conditions and after application of oxaliplatin (Fig. 4). Oxaliplatin led to an increase of the refractory time by more than threefold. This effect could only be caused by a delay of inactivation of the Na^+ channels.

Na^+ channel inactivation kinetics depend on many different factors, e.g. the subtype of Na^+ channel, metabolic factors, pH and Ca^{2+} ions. Oxaliplatin could interact with Na^+ channel inactivation by altering the pH as oxalic acid is thought to be liberated from the compound in a high Cl^- environment. This possibility seems not very likely since the pH was critically controlled in the solutions used. Furthermore, low pH is expected to rather accelerate Na^+ channel inactivation. Another possibility could be that extracellular Ca^{2+} ions are captured by the chelator oxalic acid which is liberated when oxaliplatin enters the cell. A low Ca^{2+} concentration increases neuronal excitability and is able to alter Na^+ channel inactivation. To exclude this possibility, we performed experiments in different Ca^{2+} concentrations ranging from 2.2 to 15 mM. At Ca^{2+} concentrations < 10 mM, no influence on the effect of oxaliplatin on Na^+ currents could be detected. Only higher Ca^{2+} concentrations antagonised the effect of oxaliplatin on nerve excitability (data not shown). This makes it unlikely that an altered Ca^{2+} concentration might play an important role in neurotoxicity of oxaliplatin. Interestingly, oxaliplatin affected the action potentials predominantly of myelinated A-fibres while those of unmyelinated C-fibres showed no changes (Figs. 1B and 5). A possible explanation for this difference would be that oxaliplatin acts specifically on certain isoforms of Na^+ channels which are differentially expressed in the two types of fibres. When we recorded Na^+ currents from sensory dorsal root ganglia neurons, the cell bodies of both types of fibres, oxaliplatin influenced the current in about 70% of all cells tested. The effects were characterized by a dramatic lengthening of the Na^+ currents (Fig. 6A), which was in line with our previous observation of prolonged compound action potentials in the isolated nerve. Furthermore, a small block of the maximum amplitudes and a shift of the voltage–response relationship towards more negative membrane potentials were observed. This shift, which results in a higher sensitivity of the channels to depolarizing stimuli, provides a good explanation for the enhanced amplitude of compound action potentials, for the repetitive firing in electrotonic recordings, and, as a consequence, for the hypersensitivity to minor stimuli observed in patients. A shift in the same direction and range of magnitude of tetrodotoxin-resistant Na^+ currents on rat dorsal root ganglia neurons has been reported as a result of sustained hyperglycemia and discussed as an important contribution to painful neuropathy in diabetics (Hirade et al., 1999).

The degree of effects observed in dorsal root ganglia neurons was rather variable. But differences between individual cells were not surprising, since dorsal root ganglia consist of various cell types which are responsible for different sensory modes and also differ in their pattern of expression of a variety of Na^+ channel isoforms being additionally influenced by injury and culture conditions (Waxman et al., 1994; Black et al., 1996; Cummins and Waxman, 1997; Dib-Hajj et al., 1998). There are also enzymatic modifications influencing kinetics and/or pharmacology of voltage-gated Na^+ channels (Fitzgerald et al., 1999). A subset of dorsal root ganglia neurons did not respond at all to oxaliplatin, a result that can be expected when we assume that these are the cell bodies of C-fibre axons.

Between central and peripheral neurons, there are even more distinct differences in the pattern of expressed Na^+ channel isoforms (Felts et al., 1997). In hippocampal neurons, no effect of oxaliplatin on Na^+ currents could be detected at all (Fig. 6B). Oxaliplatin discriminates between Na^+ channel isoforms and may be specific for one or more of those isoforms which are expressed mainly or only in a subset of sensory neurons. To identify these targets, recombinant isoforms expressed in a non-neuronal background have to be tested in further studies individually for their sensitivity to oxaliplatin.

It is difficult to discuss whether there is a relation between the neurotoxicity caused by cisplatin and oxaliplatin. The cisplatin neuropathy is characterized by a dose-dependent cumulative, mainly sensory, neurotoxicity. Morphological studies have shown evidence for a degenerative axonopathy. While cisplatin neuropathy is delayed and tends to worsen in the post-treatment period, the acute neurotoxicity of oxaliplatin recovers between cycles, and the cumulative toxicity tends to improve after treatment is stopped. From these observations, one can postulate that the acute neurotoxic symptoms observed with oxaliplatin might be caused by cellular mechanisms different from that of the cumulative neurotoxicity observed with both drugs, and sharing a lot of similarities. There are not enough data available showing a possible correlation be-

tween the occurrence of strong acute symptoms and late neurotoxic effects caused by oxaliplatin.

Taken together, our results identify an increased Na^+ influx due to prolonged opening of Na^+ channels as the mechanism, which causes the neuropathic symptoms observed in patients under treatment with oxaliplatin. Antagonisation of these unwanted effects with a substance which is already in clinical use would be of great help for those who suffer from severe and dose-limiting side effects during cytostatic therapy. A good candidate for such a substance is the antiepileptic drug carbamazepine which acts as a Na^+ channel blocker (Willow et al., 1984) (Fig. 5) and is also discussed for the treatment of neuropathy (Rizzo, 1997). Success seems likely since the first five patients treated with carbamazepine in a pilot study showed a dramatic reduction of side effects under therapy with oxaliplatin (C. Lersch, personal communication).

Acknowledgements

We thank Dr. R. Netzer from GENION Forschungsgesellschaft, Hamburg for help with the recordings from hippocampal neurons. This work was supported by Sanofi-Synthelabo.

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