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Behavioral and electrophysiological studies in rats with cisplatin-induced chemoneuropathy

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Abstract

Neuropathy is the chief dose-limiting side effect associated with the major classes of frontline cancer therapy drugs. Here the changes behavioral responses of rats to cutaneous mechanical and thermal stimuli occurring following treatment with cisplatin and the changes in spinal neurophysiology accompanying the development of chemotherapy-induced hyperalgesia were explored. Systemic treatment with cisplatin induced changes in both mechanical and thermal cutaneous sensory withdrawal thresholds of Sprague-Dawley rats. High doses of chemotherapy produced hypalgesia whereas lower doses produced hyperalgesia. Follow-up neurophysiological studies in rats with chemotherapy-induced hyperalgesia revealed that deep spinal lamina wide dynamic range neurons had significantly higher spontaneous activity and longer afterdischarges to noxious mechanical stimuli than wide dynamic range neurons in control rats; cisplatin administration was also associated with longer after-discharges and abnormal wind-up to transcutaneous electrical stimuli. The hyperexcitability observed during cisplatin-induced hyperalgesia is very similar to that observed in rats with hyperalgesia produced following treatment with other very diverse types of chemotherapeutic agents and similar to that observed following specific types of direct nerve injury.

Keywords

chemotherapy; neuropathy; neuropathic pain; wind-up; wide dynamic range neurons; afterdischarges

1. Introduction

Cancer treatments are almost never given at the optimal dosages or schedules to kill cancer cells. Rather treatment protocols are governed by the necessity of limiting toxicities. Protective measures have been developed for two of the major complications of chemotherapy, bone marrow suppression and renal toxicity, but the third major toxicity, debilitating and painful neuropathy remains largely unmanageable (Alberts and Noel, 1995; Cavaletti et al, 1995; Quasthoff and Hartung, 2002). Neuropathy is the chief dose-limiting side effect associated with the major classes of frontline drugs, including the taxanes, the vinca alkaloids, and the platin-based drugs, that are used against all of the most common types of cancer. The survival of hundreds of thousands of patients each year is therefore at risk due to this problem.

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Moreover, the numbness, tingling, burning pain and sensory-motor impairments characteristic of chemoneuropathy is largely refractory to treatment and often persists as a chronic condition long after treatment, thus affecting the quality of life and return to productivity in many cancer survivors (Boogerdt et al, 1990;Cata et al, 2004;Dougherty et al, 2004;Roefols et al, 1984).

A noteworthy clinical feature of chemo-related pain discovered by this laboratory is the consistency of symptoms among patients receiving very different types of agents. Patients complain of symptoms in identical distributions and describe these sensations with nearly identical word descriptors regardless of whether this was produced by treatment with taxanes, vinca alkaloids, or the proteasome inhibitor bortezomib. Quantitative sensory examination reveals nearly identical deficits in sensory function over all skin areas affected by symptoms (Cata et al, 2007;Dougherty et al, 2004;Dougherty et al, 2006). Models of chemoneuropathy in animals, like the clinical pain syndromes, are also remarkably similar in behavioral characteristics (Apfel et al, 1992;Barajon et al, 1996;Polomano et al, 2001;Tredici et al, 1998;Weng et al, 2003). Taxol and vincristine also induce essentially identical changes in the physiological properties of primary afferent fibers and spinal dorsal horn neurons. Animal models of cisplatin chemoneuropathy have been reported, but remain less well characterized than the taxane- and vinca-alkaloid models (Apfel et al, 1992;Authier et al, 2003;Bianchi et al, 2007). The platin compounds, most notably, cisplatin (cis-diaminedichloroplatinum II) are frontline treatments for many solid and hematologic neoplasms, including lung cancer, the number one cancer killer in the United States (Prestayko et al, 1979). Given the importance of not only understanding the toxicity of this key chemotherapeutic drug, but also of determining whether chemotherapy drugs as a general class show convergence in their mechanisms of neuropathic pain, the goal in this study was to explore the dosing regimen needed to induce cisplatin-chemoneuropathy in rats and to determine the resulting physiological changes occurring in spinal neurons of animals with cisplatin-induced pain.

2. Results

2.1. Behavioral experiments

2.1.1. Body weight changes—Cisplatin treatment produces a failure to normally thrive in rats (Cavaletti et al, 1992;Garcia et al, 2008;Tredici et al, 1998). This was evidenced here by a reduced rate of weight gain in the three groups of cisplatin-treated rats during chemotherapy. Failure to gain weight was evident from day 1 of treatment in the animals receiving 0.5 and 1.0 mg/kg cisplatin and only showed increases similar to the control animals following day 3 of treatment. Saline rats showed a gain in weight of $8.6 \pm 1.0\%$ over baseline in this interval whereas the rats receiving 0.5 mg/kg cisplatin showed a $-3.1 \pm 1.2\%$ change in body weight and the rats receiving 1.0 mg/kg cisplatin had a $-2.9 \pm 2.6\%$ change in body weight ($P < 0.01$). The rats treated with 0.1 mg/kg cisplatin showed a modest increase in body weight (Day 3, $4.6 \pm 0.9\%$) that was not significantly different from controls but that was significantly different from the other cisplatin-treated groups ($P < 0.001$). Cisplatin treated rats resumed a positive rate of body weight increase once chemotherapy was discontinued, though the overall rate of gain remained less than that of saline-treated rats. The rate of change in body weight among the treatment groups was comparable from day 7 to the end of the experiment.

2.1.2 Mechanical nociceptive thresholds—Baseline mechanical nociceptive thresholds to von Frey filaments were similar among rats in the four treatment groups and these thresholds remained stable and without significant change throughout the experiment in the saline and 0.1 mg/kg cisplatin-treated animals. Rats treated with 0.5 mg/kg cisplatin showed a significant decrease in withdrawal threshold that was evident following the second day of treatment and this decrease persisted until day 7 of the experiment ($p < 0.01$, Fig 1). Mechanical withdrawal thresholds in these rats then showed recovery from day 7 reaching baseline levels by day 11.

Repeated administration of 1.0 mg/kg of cisplatin did not induce increased responsiveness to the von Frey filaments, but rather a produced a decrease in responsiveness such that animals were assigned cut-off response values from days 2 to 7 ($P < 0.05$). Responses of rats to the mechanical stimuli gradually returned over the next week following chemotherapy such that normal response rates were observed by day 12.

In rats treated with 0.5 mg/kg of cisplatin, mechanical hyper-responsiveness was also manifested as an increased percentage of nociceptive responses to the von Frey filaments. At baseline both groups showed a similar percentage of paw withdrawals (cisplatin 0.5 mg/kg: $39.23\% \pm 6.06$ vs. saline: $31.0\% \pm 8.08$) to a mechanical force of 8.7 g; however, from day 2, cisplatin-treated rats had a statistically significant exaggerated response that was maximal at day 5 and remained stable until day 7 of the experiment (chemotherapy: $72.72\% \pm 8.61$ vs. saline: $39.0\% \pm 10.47$, $P < 0.01$). At day 1 of the experiment, rats injected with 1 mg/kg of cisplatin showed a statistically significant decrease in the percentage of nociceptive responses that could be interpreted as a transient hypalgesic effect ($P < 0.05$).

2.1.3 Thermal nociceptive thresholds—The administration of cisplatin at 0.1 and 0.5 mg/kg did not cause any change in the thermal nociceptive thresholds compared to saline. Only, on day 2 and 3 of the experiment did these rats show a transient and non-statistically significant increase in paw withdrawal latency (Fig 1). However, rats treated with 1.0 mg/kg cisplatin showed an increase in the paw withdrawal latencies compared to saline animals that was statistically significant from day 3 (saline: $-9.37 \pm 1.6s$ vs. cisplatin 1.0 mg/kg: $11.3 \pm 76s$, $P < 0.05$) to day 7 (saline: $8.8 \pm 1.2s$ vs. cisplatin 1.0 mg/kg: $11.6 \pm 1.3s$, $P < 0.05$) of the experiment.

2.2. Electrophysiological Studies

A total of 110 neurons were studied, 62 from saline-treated rats and 48 from cisplatin 0.5 mg/kg-treated rats. As previously reported by our group (Weng et al, 2001) and others using different models of neuropathic pain (Chu et al, 2004; Palecek et al, 1992; Palecek et al, 1993; Sotgiu and Biella, 1997) WDR neurons from neuropathic animals showed marked changes in spontaneous activity. Not only was there more frequent occurrences of spontaneous activity (73% of the WDR neurons of cisplatin rats showed spontaneous activity compared to 48% of the cells in the saline group, $p < 0.01$, Chi-Square); but as well the mean rate of spontaneous activity was higher in cisplatin-treated rats than in saline-treated rats (cisplatin 0.5 mg/kg: 2.70 ± 0.81 Hz vs. saline: 1.12 ± 0.31 Hz, $p < 0.05$).

There were also a number of significant differences in evoked activity between WDR neurons of saline and cisplatin treated rats. The evoked response to brush stimulation was lower in WDR neurons of neuropathic rats than in those of controls, but this did not achieve statistical significance ($P < 1.2$). The evoked firing to graded mechanical stimulation [von Frey filaments, venous (pressure) and arterial (pinch) clips] was significantly graded in neurons from cisplatin ($r^2 = 0.92$, $p < 0.05$) and saline rats ($r^2 = 0.98$, $p < 0.01$). However, the magnitude of the response was lower in cisplatin rats compared to vehicle animals. The mean firing rate of WDR neurons in saline-treated rats to a punctate stimulus of 14.6 g was 27.48 ± 0.31 Hz, compared to a mean firing rate of 16.41 ± 2.38 Hz in 0.5 mg/kg cisplatin-treated animals ($p < 0.05$). Similarly, with stronger stimulation (pressure) the mean firing rate of neurons in the saline rats was also higher than that of neurons in cisplatin rats, though, again not achieving statistical significance (saline: 41.72 ± 0.31 Hz vs. cisplatin: 35.18 ± 4.32 Hz, $p < 0.10$). Perhaps more importantly, hyperalgesic animals had more afterdischarges than normal animals. Afterdischarges were statistically more pronounced in cisplatin rats than in saline rats when pressure (saline: 5.03 ± 0.97 Hz vs. cisplatin: 8.49 ± 1.93 Hz, $p < 0.01$) and pinch (saline: 10.62 ± 1.69 Hz vs. cisplatin: $14.32 \pm$

1.82 Hz, $p < 0.05$) stimulations were applied to the skin (Fig 2), or when an area under the curve analysis was run for all stimuli combined ($p < 0.001$).

The latency times for the A- and C-fiber responses and the mean number of spikes in the A-fiber latency period after the first electrical cutaneous stimulus did not differ between WDR cells of the different groups. Electrical stimulation at 0.3 and 1 Hz caused comparable wind-up responses in both groups of animals. However, repeated stimulations at 0.1 Hz caused an abnormal and strong windup response in WDR neurons of cisplatin-treated rats that was significantly larger than that in control rats from the ninth electrical stimulus to the last (16th) consecutive stimulus ($P < 0.01$) (Fig 3).

3. Discussion

There were two broad sets of new findings in this work. The first is in regards to the behavioral studies. The data presented here bridge the discrepant findings of the two previous reports on cisplatin-induced neuropathy. The earliest study found that cisplatin treatment produced an impairment of heat sensitivity in mice (Apfel et al, 1992), whereas the second report showed mechanical and thermal (cold) hyperalgesia following cisplatin treatment in rats (Authier et al, 2003). The high dose of cisplatin used here produced elevated thermal and mechanical withdrawal thresholds consistent to the findings in the mice where relatively high doses of cisplatin were also used. Hypoalgesia does not appear to be explained by motor impairment as cisplatin-treated rats did not have deficits in rotarod performance (Garcia et al, 2008). On the other hand, the lower dose schedule used here produced mechanical hyperalgesia very similar to that in the previous study using rats. Thus, these findings appear to resolve the earlier discrepancies as simply an issue of dose, wherein low doses produce hyperalgesia and higher doses producing hypoalgesia. The only minor differences in our results in comparison to some of the earlier work is that here the onset of effects was earlier and at a lower cumulative dose of cisplatin. It is unclear what might explain these subtle differences in behavioral effects, particularly given the fact that similar results were obtained by both groups for the effects of cisplatin on body weight. This suggests that at least the areas of the central nervous system regulating feeding and body weight were similarly affected by the treatments in the two labs. For the time being it is concluded that the differences in behavioral effects are due the commonly seen behavioral variation among differing animal strains.

The second set of new findings concerns the physiological changes occurring in the spinal cord following systemic chemotherapy and the induction of chemo-neuropathy related hyperalgesia. There are two major points in this regard. First, the physiological changes observed in spinal WDR neurons of rats with cisplatin-induced hyperalgesia turn out to be very similar to those reported in animal models of vincristine- and paclitaxel-induced hyperalgesia and other models of neuropathic pain (Cata et al, 2004; Pertovaara et al, 1997; Weng et al, 2003). Mechanical cutaneous stimulation of the hind paw of cisplatin-treated animals did not evoke neuronal discharge higher than that of controls; in fact, the evoked activity was lower. This finding is consistent with an acute effect of cisplatin in reducing voltage-gated calcium currents in dorsal root ganglion neurons of rats (Tomaszewski and Büselberg, 2007), but is also clearly not in agreement with the drop in the paw withdrawal threshold seen in the behavioral experiments. A similar mismatch between the animal behavior and the electrophysiological findings has been observed in other models of neuropathic pain (Hao et al, 2004; Laird and Bennett, 1993). It is possible that changes in synchronization of spinal dorsal horn neurons and relative effects of descending regulatory systems induced by anesthetic agents could be responsible for these findings and hence the physiology in awake animals may differ (Pertovaara et al, 2001). It is also possible that cisplatin-treated animals have ongoing paresthetic sensations, such as tingling and numbness, similar to those manifested by patients after receiving chemotherapy that result in enhanced vigilance of the animals to peripheral stimuli. This

possibility is supported by the observation of higher spontaneous activity and the exaggerated after-discharges in WDR neurons in neuropathic rats than controls. This possibility might suggest that the behavioral responses in fact reflect a learned avoidance response wherein the animals are attempting to avoid a prolonged or exaggerated dyesthesia as opposed to a nociceptive withdrawal per se. However, it should be noted that cisplatin-treated rats did not exhibit autotomy as a sign of spontaneous pain. Additionally, restricted behavioral testing of a small cohort of chemotherapy-treated animals only at baseline and then at the expected peak time of hyperalgesia revealed lowered withdrawal thresholds as in the animals that received the full behavioral testing paradigm as illustrated in Figure 1.

The second major point of significance in the physiological data is the revelation of marked similarities between the physiological changes in chemoneuropathy as compared to models of direct nerve injury-related pain. Elevated background activity in the context of normal evoked responses seen in animals with cisplatin, vincristine and taxol-neuropathy were also in WDR neurons of rats with diabetic painful neuropathy, and chronic constriction injury of the sciatic nerve (Cata et al, 2004; Laird and Bennett, 1993; Palecek et al, 1992; Pertovaara et al, 2001; Weng et al, 2003). Increased spontaneous activity could reflect a loss of inhibition exerted by large myelinated fibers on dorsal horn neurons. This possibility is supported by the decrease in numbers and function in large sensory fibers observed in the sciatic nerve and dorsal root ganglion of cisplatin-treated humans and animals (Barajon et al, 1996; Roefols et al, 1984). However, the transient characteristics of the mechanical allodynia found here would argue against a permanent loss of sensory or functional reorganization of afferents within the spinal cord (Devor, 1988; Fitzgerald et al, 1990). It is thus also possible that increased activity in damaged peripheral afferents could be responsible for augmented background activity and central sensitization. Cisplatin accumulates in high concentrations in the dorsal root ganglia (DRG), and it has been reported that in rats with spinal nerve ligation and neuropathic pain the spontaneous activity of DRG cells is increased within 24 h of the nerve damage (Liu et al, 2000). Therefore, it seems possible that early alterations in cellular metabolism, gene expression, protein synthesis and axonal transport due to cisplatin accumulation and possible activation of pro-apoptotic pathways could drive altered function and excitability of DRG neurons (McKeage et al, 2001).

Another point of convergence regards the changes in afterdischarges occurring in the chemoneuropathy as well as the direct nerve injury models. Afterdischarges due to noxious and non-noxious stimulation were shown here to be prolonged in the WDR neurons of cisplatin treated rats and has also been observed in paclitaxel- and vincristine-treated rats, as well as in other models of nerve-injury related pain (Cata et al, 2004; Hao et al, 2004; Pertovaara et al, 2001; Sotgiu and Biella, 1997; Weng et al, 2003). Increased afterdischarges may again reflect a loss of inhibitory tone in the dorsal horn that could be as a consequence of loss of large myelinated-fiber input. However, a potentially intriguing alternate explanation is that cisplatin treatment induces a down-regulation of the expression of spinal glutamate transporters, thus resulting in slowed clearance of synaptically-released neurotransmitter. The precedent for this possibility has already been established in that the glutamate transporters GLAST, GLT-1 and EAAC-1 are all reduced in the spinal dorsal when measured at the peak of hyperalgesia induced by treatment with taxol (Cata et al, 2006).

In conclusion, we have demonstrated that rats treated with 0.5 mg/kg of cisplatin developed transient mechanical allodynia and hyperalgesia in addition to high spontaneous activity and prolonged afterdischarges in WDR neurons of the dorsal horn of the spinal cord. These physiological changes are very similar to that observed among spinal neurons of rats with diverse models of chemotherapy-induced pain as well as with those of rats with direct nerve injuries. The major issue to be resolved is the initiating locus for the changes in spinal physiology in the chemoneuropathy animals. Given that chemotherapy drugs poorly penetrate

to the CNS (Kellie et al, 2002), but that these drugs do penetrate into dorsal root ganglion cells and peripheral axons (Cavaletti et al, 2000) it suggests that effects initially occurring in peripheral nerve endings initiate the pronounced changes in spinal physiology. Further study will be required to elucidate the basis of this transition.

4. Experimental Procedure, Acknowledgements, References

Thirty six (36) male Sprague-Dawley rats weighing 175–225 grams were used in accordance with the guidelines of the National Institute of Health and the International Association for the Study of Pain and with the consent of the Institutional Animal Care and Use Committee of the MD Anderson Cancer Center. The animals were individually housed on a 12:12 light-dark cycle with food and water ad libitum. The rats were divided into four equal treatment groups. The first three groups received daily intraperitoneal injections of 0.1 mg/kg, 0.5 mg/kg, or 1.0 mg/kg cisplatin (Sigma) that was dissolved in saline and injected in a total volume of 1.0 ml over 3 consecutive days. The fourth group received daily injections of 1.0 ml saline to provide a comparison group. The dosages of cisplatin were selected based on preliminary dose finding studies and based on the historical use of cisplatin in tumor-bearing rodents (Bencokova et al, 2008).

4.1 Behavioral experiments

4.1.1 Mechanical Withdrawal Threshold—Mechanical withdrawal thresholds were determined by an investigator blinded to treatment conditions. Each animal was loosely restrained beneath a plastic box on a metal mesh and allowed to acclimate for at least 15 minutes once per day for one week prior to further testing. On the first testing day the animals were reintroduced to the testing environment, allowed to accommodate and then von Frey filaments (bending force 0.06 – 14.6 g) were presented alternately from below to the mid-plantar surface of each hind paw. A nociceptive response was defined as brisk paw withdrawal following von Frey filament application. Each hind paw was stimulated on five occasions and the 50% withdrawal threshold was defined as the lowest bending force that evoked at least total five paw withdrawals (Weng et al, 2003). Mechanical withdrawal thresholds were measured at baseline prior to any chemotherapy, daily before each round of chemotherapy and then on alternate days thereafter until withdrawal threshold recovered to baseline levels or the animals entered the neurophysiology studies.

4.2.2. Thermal Withdrawal Threshold—Paw withdrawal latency to radiant heat was assessed as previously described (Hargreaves et al, 1988). Each animal was loosely restrained beneath a plastic box on a glass surface and allowed to acclimate for at least 15 minutes once per day for one week prior to behavioral testing. Testing comprised alternate presentations of radiant heat from below to each hind paw focused on the mid-plantar surface. The heat source cut-off automatically with paw withdrawal and the latency recorded. Paw withdrawal latency was defined as the mean of three trials applied to each paw. A cutoff stimulation time of 24 s was used in the event of no movement to avoid skin damage (Weng et al, 2003).

4.2. Electrophysiological studies

4.2.1. Animal preparation—Animals were anesthetized with urethane (1.4–1.6 g/kg). The right jugular vein and carotid artery were cannulated for fluid and drug administration and measurement of blood pressure, respectively. A tracheal cannula was inserted and artificial ventilation initiated. For assisted mechanical ventilation, rats were paralyzed with pancuronium bromide (2 mg/kg, i.v.) and ventilated with 95% oxygen and 5% CO₂. Anesthesia depth was determined by constancy in pupil size and absence of reflex response to noxious stimuli prior to administration of the muscle relaxant and later monitored by stability of heart rate and blood pressure autonomic reflexes to noxious stimuli. End tidal CO₂ concentration

was monitored using a small-animal CO₂ analyzer and maintained between 3.5 and 4.5%. Rectal temperature was maintained at 36–38 °C by means of a thermostatically controlled heating lamp. The animal was positioned in a spinal frame and the thoracolumbar spine stabilized with vertebral clamps. Spinal vertebrae T10–L2 were removed and the dura mater opened with fine surgical forceps. The spinal cord was protected from desiccation below an agar pool filled with artificial cerebrospinal fluid.

4.2.2. Extracellular neuronal recordings—Dorsal horn neurons located 300–900 µm below the dorsal surface of the spinal cord in segments L4–L5 were recorded extracellularly using paralyne-coated tungsten microelectrodes (Microprobes, Inc, resistance, 1.4–1.8 MΩ) that were advanced through the dorsal horn with a hydraulic microdrive. Cells were isolated by gentle tapping of the skin. Spike waveforms were monitored on an oscilloscope, amplified and sent to a data analysis system (CED 1401 for PC, Cambridge) for data collection using Spike-2 software.

WDR neurons were defined as cells that responded to both natural non-noxious (camel brush) and noxious (compression) skin stimulation. The background activity was monitored and recorded over at least 2 min for each cell prior to any other stimuli. Graded natural noxious mechanical stimulation were then applied to the center of the receptive field using von Frey filaments as in the behavioral studies, repeated stroking with a soft camel hair brush, and the application of venous (VC: 1.3N, pressure) and arterial (AC: 3N, pinch) clips. Each mechanical stimulus was applied for 5 s, with an interstimulus interval of 10–30s (depending on the occurrence of afterdischarges, see below) (Weng et al, 2001). Electrical stimuli were delivered using a pair of fine intradermal needles inserted about 1.5 mm apart into the center of the receptive field area. The responses to electrical stimuli 200µsec in duration and 1.0mA in amplitude were collected at multiple stimulus frequencies (0.1, 0.3, and 1 Hz). The Aδ and C components of the evoked electrical response were considered as the action potentials that occurred between 0 and 20 ms and 90 and 300 ms after the electrical stimulus, respectively (Chapman et al, 1998). Wind-up (Mendell, 1966) in action potentials was calculated as the percentage change in discharges at C-fiber latencies for each stimulus pulse compared to the first stimulus response (Weng et al, 2001).

4.3. Statistical analysis

Behavioral data were analyzed using the Mann-Whitney U test. Neuronal responses to all stimuli were analyzed off line. Spikes were discriminated from background (and other units if present) by use of threshold windows and template-matching software (Spike 2). The spikes evoked during the 5 s application of the natural stimuli and spikes occurring for the next 5 s following removal of the natural stimuli (after-discharges) were quantified. The spikes evoked by the electrical stimuli were separated into three components, an early A-fiber evoked response, a late C-fiber evoked response, and after-discharges occurring at delays of 1 s to the end of 10 s from the stimulus onset. These data were compared using two way analysis of variance (ANOVA) with repeated measurements followed by Tukey post hoc analysis or by *t*-test where appropriate. Change in stimulus–response function was determined by ANOVA with repeated measures design. *P* values of less than 0.05 were considered significant. Change in distributions of spontaneous activity, afterdischarge, etc, were compared using Chi-Square. All data are presented as the mean ± SEM unless otherwise indicated

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Figure 1.

A. The upper panel shows the 50% withdrawal threshold to mechanical stimulation of the hindpaws in saline (open circles), and cisplatin-treated animals (filled symbols). Rats receiving 0.1 mg/kg cisplatin (filled triangles) showed no changes in responses to mechanical stimulation. Rats receiving 0.5 mg/kg cisplatin treatment (filled circles) showed a significant decrease in the 50% withdrawal threshold that was evident at day 2 and that continued to day 11. Finally, rats receiving treatment with 1.0 mg/kg cisplatin (filled squares) showed an increase in 50% withdrawal threshold from days 1 to 7. B. The lower panel shows the mean paw withdrawal latency to thermal stimulation over time with chemotherapy. The only differences that were found occurred in the rats receiving 1.0 mg/kg cisplatin treatment where

a significant prolongation of withdrawal latency occurred from days 3 to 7. Stars indicate differences between labeled data points and saline controls. One star , $p<0.05$; two stars, $p<0.01$.

A. Representative Cells

B. Mean Acute Evoked Responses

C. Afterdischarges

Figure 2.

Neurophysiological findings from rats treated with saline and rats with hyperalgesia following treatment with 0.5 mg/kg cisplatin are illustrated here. A. Representative analog recording from a WDR neuron in a saline-treated rat (top line) showing typical responses to mechanical brushing of the skin (Brush), the application of various strengths von Frey filaments (strength shown in grams), and the application of firm non-painful skin compression (Pressure) and painful compression of the skin (Pinch) using two sizes of arterial clips. The second line in A shows representative analog recordings of the responses of a WDR cell from a rat with cisplatin-induced hyperalgesia to the same stimuli. Note that cisplatin-treated rats had increased spontaneous activity and prolonged afterdischarges after pressure and pinch. B. The bar graphs

at the bottom of the figure show the mean number of spikes of WDR cells from saline (open bars) and from cisplatin-treated rats (closed bars) to the mechanical stimuli. C. The mean number of afterdischarges present after each mechanical stimulus. The bars are coded as in B. Stars indicate statistical differences ($p < 0.05$).

Figure 3.

A. Representative analog recordings show the acute responses of WDR cells from saline and cisplatin-treated rats to repetitive transcutaneous electrical stimulus at an interval stimulus of 0.1Hz. Note that repeated stimuli at this frequency show little change in the WDR cell from a saline-treated rat (left column) while a WDR cell from a neuropathic animal showed marked wind-up. B. The line/scatter graphs at the bottom depict the mean normalized response of WDR neurons from saline-treated (open circle) and cisplatin-treated rats (closed circle) to 16 consecutive electrical stimuli delivered at 0.1Hz in reference to the first pulse delivered at each intensity.