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Impaired bronchomotor responses to field stimulation in guinea-pigs with cisplatin-induced neuropathy

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Abstract

Pre-treatment with cisplatin (3 mg/kg) i.p. once a day over 6 days induced sensory neuropathy as confirmed by femoral nerve conduction velocity test and significantly decreased contractions induced by electrical field stimulation (100 stimuli, 20 V, 0.1 ms, 20 Hz) in isolated main bronchial rings from guinea-pigs. The field stimulation-induced non-adrenergic, non-cholinergic (NANC) relaxations, however, were amplified in rings from animals with cisplatin neuropathy. The NANC relaxation response was completely blocked by 30 μM N^G-nitro-L-arginine methyl ester in preparations from both control and cisplatin-treated animals. Superoxide dismutase (40 units/ml) was without effect on NANC relaxation in control rings, however, it substantially decreased NANC relaxation in preparations from animals with cisplatin neuropathy. These results show that cisplatin-induced sensory neuropathy is accompanied by attenuation of neural bronchoconstriction and an enhanced NANC relaxation. The latter is in part attained by an increased peripheral superoxide production.

Keywords: Cisplatin; Sensory neuropathy; Superoxide production; Peroxinitrite

1. Introduction

Capsaicin-sensitive primary afferent neurons have been shown to play a major modulatory role in the regulation of bronchomotility (Coburn and Tomita, 1973; Barnes, 1990). Regarding the neurotransmitters involved, most evidence favours a role for calcitonin gene-related peptide (CGRP), substance P, neurokinin A, somatostatin and possibly nitric oxide (NO) (Nijkamp and Folkerts, 1995; Szolcsanyi, 1996). It is therefore not surprising that diseases or pharmacologic manoeuvres result in the damage of these neurons such as progression of Type I diabetes or treatment with capsaicin impair bronchomotility (Helander, 1958; Casaco et al., 1989; Perretti and Manzini, 1993).

Cisplatin is a chemotherapeutic agent used for the treatment of several types of cancer. Unfortunately, cisplatin's therapeutic potential is limited by divers adverse effects such as myelosuppression, nephrotoxicity, ototoxicity and neurotoxicity (Stewart et al., 1987; Fillastre and Raguenez, 1989; Ozols and Young, 1995). The drug-induced neurotoxicity is characterized by a decrease in sensory nerve conduction velocity and a deficiency in axonal transport of sensory neuropeptides such as that of CGRP, substance P, galanin and somatostatin (Barajon et al., 1996). However, to the best of our knowledge, no study has been conducted as to whether a sensory-effector dysfunction occurring in cisplatin-neuropathy is reflected in changes in bronchial reactivity. The present work was therefore concerned with the possibility that neurotoxic cisplatin doses may deteriorate non-adrenergic, non-cholinergic (NANC) contractile responses to field stimulation in isolated bronchial preparations of the guinea-pig.

2. Methods

2.1. Ethics

The experiments performed in the present work conform to European Community guiding principles for the

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care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical boards of Medical Universities of Pecs and Debrecen, Hungary.

2.2. Treatment groups

The study was carried out with 15 male guinea-pigs weighing 350–400 g. They were housed in an animal room (12-h light/dark periods a day, temperature of 22–25°C, humidity of 50–70%) with two animals per pen fed commercial laboratory chow and tap water ad libitum. The animals were random divided into two experimental groups. Control: animals treated with the solvent for cisplatin (1 ml isotonic NaCl) with 75 mg/kg mannitol i.p. once a day over 6 days; Cisplatin-treated: animals treated with 3 mg/kg cisplatin with 75 mg/kg mannitol i.p. once a day over 6 days.

2.3. Isometric tension measurements

After exsanguination, rings from the main bronchi (3 mm) were mounted horizontally on two small L-shaped glass hooks of which one was connected to a force transducer for measurement of isometric tension. The experiments were carried out in thermostatically controlled (37 ± 0.2°C) organ bath (5 ml) (TSZ 02, EXPERIMETRIA UK, London, England) containing Krebs solution. The organ fluid was gassed with 95% O_2 and 5% CO_2 to maintain pH at 7.40 ± 0.05. Neural effects on contractile activity of the segments were studied by means of field stimulation (100 stimuli at 20 V, 0.1 ms and 20 Hz at an initial tension of 15 mN). The rings were prepared from six animals in each group. To study whether the field stimulation protocol applied was selective for nerve-mediated responses, some rings underwent a period of 10-min pre-incubation with tetrodotoxin, a fast sodium channel blocker. Stimulation with these parameters failed to elicit any contractile response in the presence of 1 μM tetrodotoxin.

2.4. Nerve conduction velocity studies

This series of experiments was carried out to verify/exclude sensory neuropathy. Left saphenous nerve conduction velocity was determined in animals from both groups as described (Nemeth et al., 1999b). In brief, in artificially ventilated animals anaesthetized with sodium thiopentone (30 mg/kg i.p.) the nerve was prepared, cleaned of fat ad adhering connective tissues and strains of square-wave (500 μs) constant voltage stimuli were applied through pairs of platinum electrodes (EXPERIMETRIA UK) placed as high as possible. Another pair of electrodes was applied 2 cm distal to the stimulating electrodes for recording the summation action potentials evoked by the proximal stimulation. The time lags between stimulation and the appearance of corresponding 'A' and 'C' signals were determined for calculation of average conduction velocity by dividing the inter-electrode distance by the interval between the end of the stimulatory impulse and the appearance of the 'A' and 'C' signals (Janig and Lisney, 1989).

2.5. Experimental protocol

The animals in each group were anaesthetized for nerve conduction velocity studies 24 h after the last cisplatin/vehicle dose. Bronchial rings were then prepared from the same animals for isometric tension measurements. After a 60-min period of equilibration, the rings were subjected to the field stimulation protocol. Two series of stimuli were applied to study the reproducibility of the responses. The rings were then incubated with guanethidine (4 μM) and atropine (1 μM, 'NANC' solution) and the field stimulation protocol was repeated. Subsequently, the preparations were exposed to additional incubation with 30 μM N^G-nitro-L-arginine methyl ester, a NO synthase inhibitor (Rees et al., 1990) followed by the field

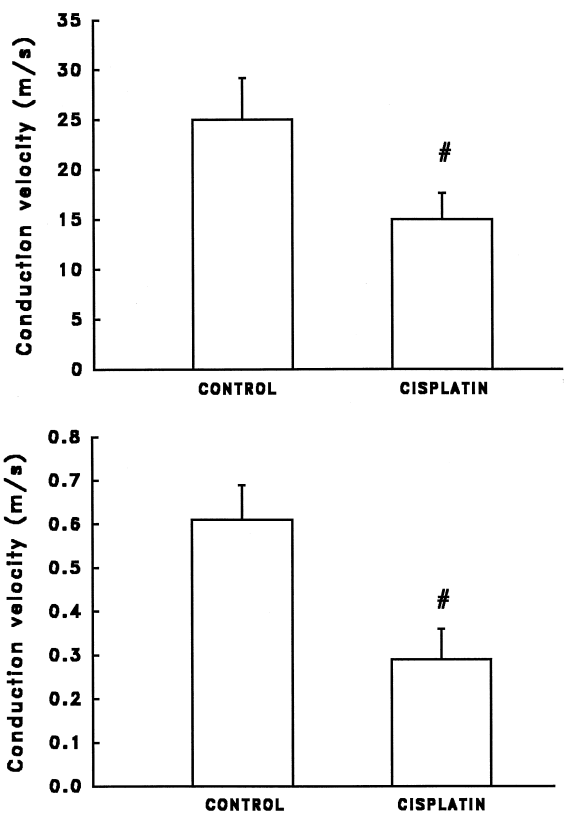
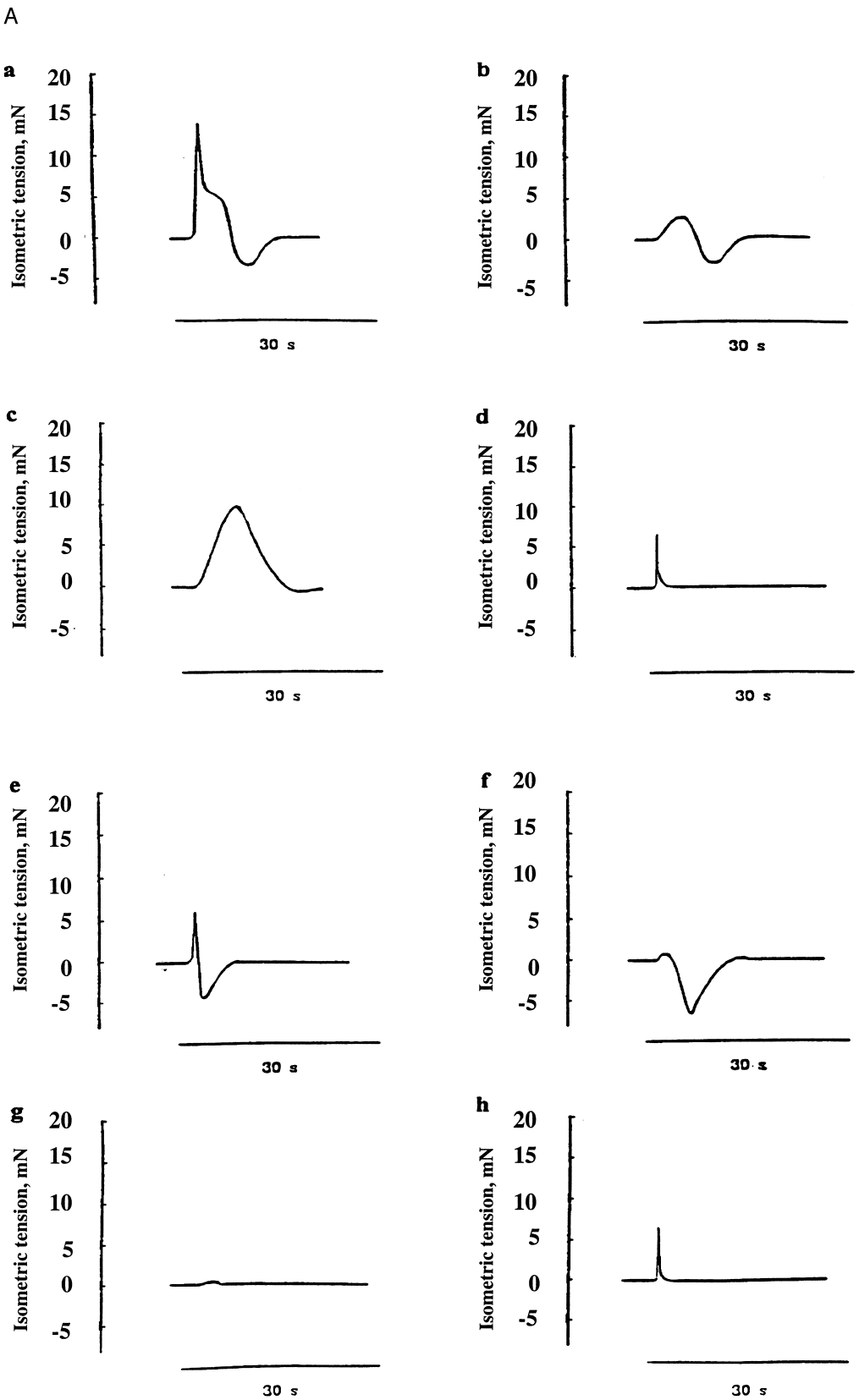


Fig. 1. Cisplatin-induced decrease in nerve conduction velocity in fast conducting myelinated (left panel) and slow conducting unmyelinated C (right panel) fibres of the femoral nerve. Measurements were done 24 h after a series of six intraperitoneal injections of cisplatin (3 mg/kg) and/or its vehicle once a day. The data are means ± S.D. obtained with six animals per group. #: significantly different from control at $P < 0.05$.

stimulation manoeuvre again. After completion of these interventions, the preparations were extensively rinsed until the initial contractile responses to field stimulation were re-gained. Separate rings were used for studies with cap-



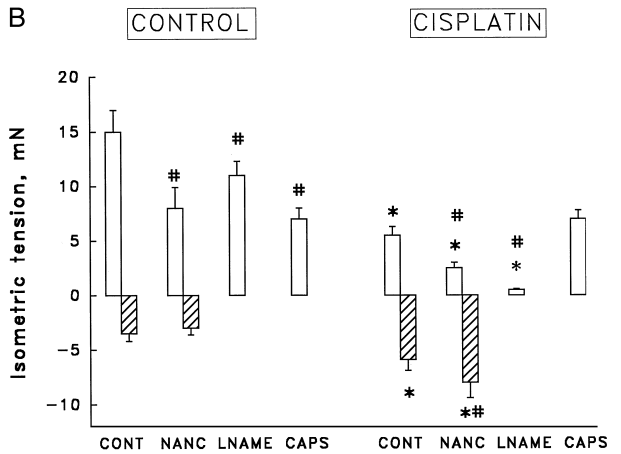


Fig.2. Original tracings (A) and summarizing data (B) representing changes in isometric tension in bronchial preparations from control (first and second rows) and cisplatin-treated (third and fourth rows) guinea-pigs in response to field stimulation (20 V, 0.1 ms, 20 Hz, 100 stimuli); (a) rings from control animals in Krebs solution, (b) rings from control animals incubated in 'NANC' (4 μM guanethidine and 1 μM atropine) solution, (c) 'NANC' solution plus 30 μM N^G-nitro-L-arginine methyl ester (L-NAME), (d) after capsaicin (100 μM), (e) rings from cisplatin-treated animals in Krebs solution, (f) rings from cisplatin-treated animals incubated in 'NANC' solution, (g) rings from cisplatin-treated animals in 'NANC' solution plus 30 μM L-NAME; h) rings from cisplatin-treated animals after capsaicin (100 μM). In part B, open columns represent contractions, hatched columns show relaxations. The data are means ± S.D. obtained with six preparations per group. #: significantly different from CONT (Krebs solution only) at $P < 0.05$; * control vs. corresponding cisplatin-treated at $P < 0.05$.

saicin and superoxide dismutase, respectively. In order to determine whether capsaicin-sensitive sensory neurons contributed to the electrically evoked contractile responses, the rings were exposed to capsaicin (100 μM) over a period of 30 min after the control stimulation had been accomplished. This was followed by a 30-min washout period and the stimulation protocol was applied again. To study whether removal of the superoxide anions influenced the NANC contractile responses, rings from both groups incubated in NANC solution were exposed to the field stimulation protocol in the presence or absence of superoxide dismutase (40 units/ml).

2.6. Drugs and chemicals

All drugs and chemicals applied have been purchased from Sigma (St Louis, Mo) except capsaicin which was from Fluka, Buchs, Switzerland). Guanethidine, atropine and N^G-nitro-L-arginine methyl ester were dissolved in Krebs solution, capsaicin was dissolved in ethanol diluted with Krebs solution.

2.7. Statistics

The results expressed as means ± S.D. were analyzed with one-way analysis of variance followed by a modified *t*-test for repeated measures according to Bonferroni's method (Wallenstein et al., 1980). Changes were considered significant at $P < 0.05$.

3. Results

3.1. Exclusions

Four cisplatin-treated animals had to be excluded from the experiments, two of them as cisplatin failed to produce any decrease in nerve conduction velocity in either A or C fibres, one because of respiratory insufficiency due to pneumonia and one because of the development of extended skin lesions.

3.2. Body weight and rectal temperature

Body weight decreased from pre-treatment value of 381 ± 41 to 308 ± 31 g ($P < 0.05$) in the cisplatin-treated animals. The 'control' guinea-pigs did not exhibit any change in body weight during the treatment period. Rectal temperature did not change in animals in either group.

3.3. Nerve conduction velocity

Fig. 1 shows the cisplatin-induced decrease in nerve conduction velocity in fast conducting myelinated (A fibres in Fig. 1, left panel) and slow conducting unmyelinated (C fibres in Fig. 1, right panel) fibres. At a stimulation intensity suprathreshold for A (0.5 V, 5 Hz) or C (3 V, 5 Hz) fibres, conduction velocity significantly decreased in cisplatin-treated animals.

3.4. Changes in isometric tension in response to field stimulation

Field stimulation in tracheal rings from 'control' animals evoked a biphasic response, the first contractile component of which comprised an initial fast and a subsequent slow reaction. This two-phase contraction was followed by a relaxation response. The fast contractile component was abolished in NANC solution, whereas the slow one disappeared in tissues pre-exposed to 100 μM capsaicin. The NANC relaxation was blocked after a 30-min incubation with 30 μM N^G-nitro-L-arginine methyl ester (Fig. 2).

In rings from animals treated with cisplatin, both the amplitude and duration of the field stimulation-induced contractile phase were significantly attenuated. However, the amplitude of the relaxation phase sensitive to N^G-nitro-L-arginine methyl ester was augmented. The contractile 'spike' left by pre-incubation with capsaicin, however, was similar in preparations from either group (Fig. 2).

The NANC contractile responses were not influenced by superoxide dismutase in preparations from the control

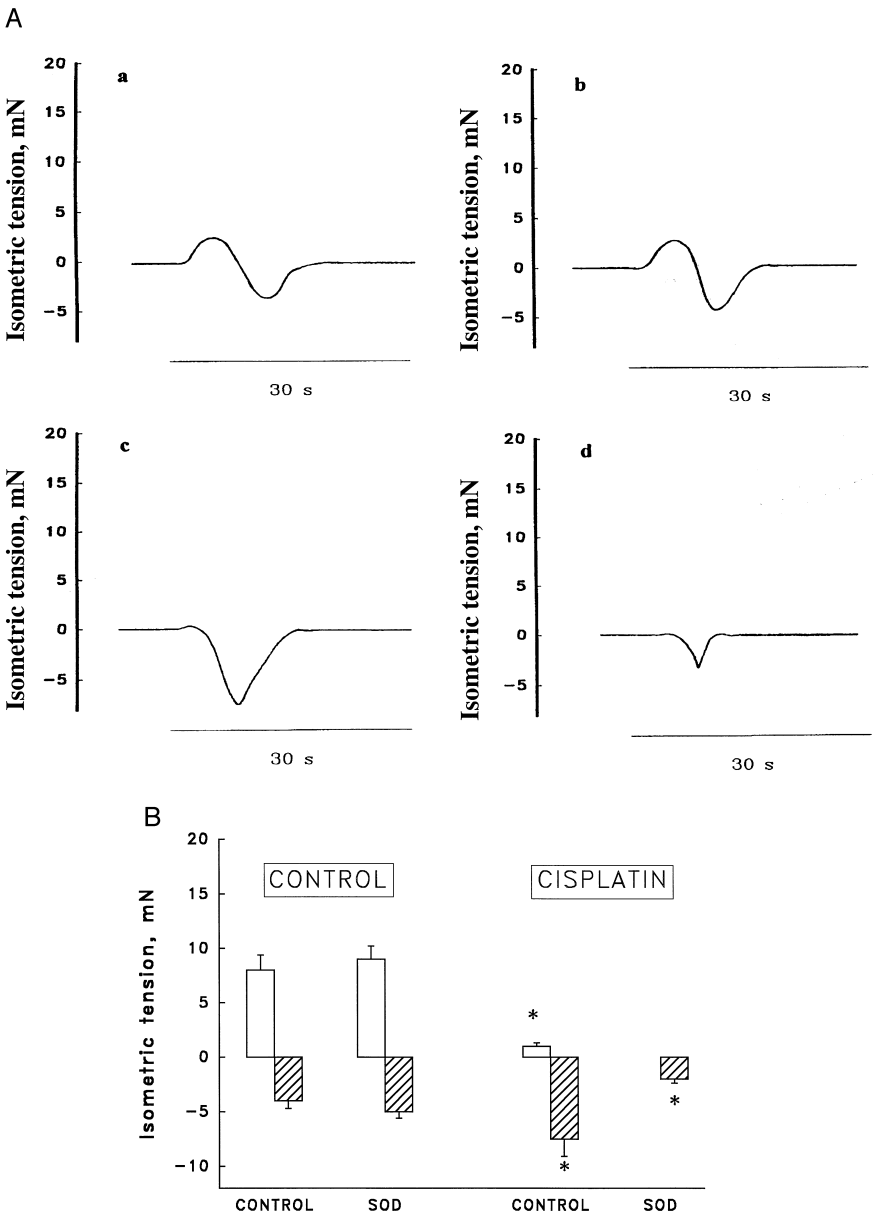


Fig. 3. Original tracings (A) and summarizing data (B) indicating changes in tension in bronchial rings from control (first row) and cisplatin-treated (second row) guinea-pigs in response to field stimulation (20 V, 0.1 ms, 20 Hz, 100 stimuli). (a) NANC solution; (b) NANC solution with 40 units/ml superoxide dismutase; (c) rings from cisplatin-treated animals, NANC solution; (d) NANC solution with 40 units/ml superoxide dismutase. In part B, open columns represent contractions, hatched columns show relaxations. The data are means ± S.D. obtained with six preparations per group. * control vs. corresponding cisplatin-treated at $P < 0.05$.

animals. In preparations from the cisplatin-treated group, however, the NANC relaxation phenomenon was substantially reduced in the presence of superoxide dismutase (Fig. 3).

4. Discussion

These results indicate that cisplatin-induced sensory neuropathy is associated with a reduced contractile response to field stimulation in isolated bronchi of the guinea-pig. This attenuated reaction can be attributed to a deficiency in sensory-effector nerve function since it was the atropine-resistant and capsaicin-sensitive section of the contractile response, which was deteriorated by cisplatin. Nevertheless, the NANC relaxation was much more pronounced in preparations from the cisplatin-treated group than in those from the control animals. Since the NANC relaxation response was completely blocked by N^G-nitro-L-arginine methyl ester in either group, it seems to be essentially nitrergic in nature. However, the nitrergic relaxation response was significantly attenuated by scavenging the superoxide anions with superoxide dismutase in preparations from the cisplatin-treated animals contrary to that

seen in 'control' rings in which superoxide dismutase was without effect.

The cisplatin-treated guinea pigs exhibited characteristic neurophysiological features of sensory neuropathy in that a significant decrease in conduction velocity occurred in both fast and slow conducting sensory nerve fibres. However, it has been found that the dorsal root ganglia cells serve as the primary targets for cisplatin to induce peripheral neuropathy (Gispen et al., 1992; Barajon et al. 1996; Cavaletti et al., 1998). It is therefore not surprising that sensory neuropathy highlights neurotoxicity produced by cisplatin (Gispen et al., 1992; Tredici et al., 1994). Neuromorphologic studies by Barajon et al. (1996) on cisplatin-induced changes revealed an accumulation of sensory neuropeptides (CGRP, substance P, galanin and somatostatin) in dorsal root ganglia with much more severe histological alterations in ganglionic cells than those seen in peripheral fibres. These studies also suggested an impaired axonal transport of sensory neuropeptides by cisplatin.

Bronchial preparations are known to be densely innervated by CGRP, substance P, neurokinin A and somatostatin containing unmyelinated afferents (Lundberg et al., 1983, 1984) which originate from the vagus nerve with cell bodies in the jugular, nodose and dorsal root ganglia (Springall et al., 1987). The sensory-effector function of these nerves is underlain by the release of these neuropeptides from the nerve terminals in response various challenges such as an increase in extracellular K^+ concentration, acidosis or electrical stimulation (Szolcsanyi, 1996; Ferdinandy et al., 1997; Szolcsanyi et al., 1998). Since sensory neuropeptides play a major modulatory role in both NANC contractions and relaxations of the bronchial muscle, it is possible that a decreased availability of excitatory neuropeptides to be released by field stimulation is responsible for the feeble NANC contractions in preparations obtained from cisplatin-treated animals similar to neuropathy induced by insulin deficient diabetes (Nemeth et al., 1999a). However, the unexpected finding of the augmented nitrergic relaxation in rings from the cisplatin-treated group can hardly be explained on the basis of a decreased availability of transmitter/transmitter producing apparatus. Of course, attenuation of excitatory impulses may be of significant modifying effect but taking the difference in sensitivity of preparations from control or cisplatin-treated animals to superoxide dismutase into account, mechanisms other than simple negative synergism may be suspected. What is more likely is that NO release in response to electrical stimulation leads to peroxinitrite formation in the presence of increased amounts of superoxide anions (Beckman et al., 1990) the production of which is characteristic of cisplatin-induced neuropathy (Matsushima et al., 1998; Fukaya and Kanno, 1999). This is suggested by the facts that NANC relaxation is completely blocked by NO synthase inhibition irrespective of the presence or absence of cisplatin neuropathy. On the other hand, the control preparations were resistant to superoxide dismutase, whereas the superoxide scavenger significantly attenuated the NANC response in rings from the neuropathic guinea-pigs.

In summary, the results presented show that cisplatin-induced sensory neuropathy is accompanied by attenuation of the effector function of sensory nerves regulating bronchial excitability. The decrease in contractile responses associated with an augmented NANC relaxation response possibly underlain by an increased peripheral superoxide production in the neuropathic animals. This calls attention to widespread alterations in neural regulatory processes in cisplatin-induced neuropathy and to the possible benefit from cisplatin therapy in asthmatic patients suffering from neoplastic diseases.

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