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Decreased sensory neuropeptide release in isolated bronchi of rats with cisplatin-induced neuropathy

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Abstract

We studied if attenuated neurogenic bronchoconstriction was associated with a change in sensory neuropeptide release in preparations from rats with cisplatin-induced neuropathy. Electrical field stimulation (100 stimuli, 20 V, 0.1 ms, 20 Hz) induced an increase in the release of somatostatin, calcitonin gene-related peptide (CGRP) and substance P determined by radioimmunoassay from baseline 0.18±0.01, 0.17±0.01 and 0.86±0.02, to 0.59±0.02, 1.77±0.04 and 5.96 fmol/mg wet tissue weight, respectively, in organ fluid of tracheal tubes from rats. This was significantly attenuated to post-stimulation values of 0.36±0.02, 0.45±0.02, 4.68±0.24 fmol/mg wet tissue weight for somatostatin, CGRP, and substance P, respectively, with a significant decrease in field stimulation-induced contraction of bronchial preparations from animals 11 days after a 5-day treatment period with cisplatin (1.5 mg/kg i.p. once a day). The cisplatin-treated animals developed sensory neuropathy characterized by a 40% decrease in femoral nerve conduction velocity. The results show that a decrease in tracheo-bronchial sensory neuropeptide release associates with feeble bronchomotor responses in rats with cisplatin-induced sensory neuropathy.

Keywords: Cisplatin; Sensory neuropathy; Neuropeptide release

1. Introduction

It has been shown that neurotoxic doses of cisplatin impair contractile responses to electrical field stimulation in isolated bronchial preparations of the guinea pig (Szilvassy et al., 2000). The attenuated neurogenic bronchoconstriction in cisplatin-induced neural dysfunction was found to be underpinned by altered non-adrenergic, non-cholinergic (NANC) responses, with a predominant deficiency in sensory-effector function of capsaicin-sensitive bronchial nerve fibres (Coburn and Tomita, 1973; Barnes, 1990), similar to that found in association with diabetic neuropathy (Szilvassy et al., 2002). The sensory-effector function of these nerves is known to be executed by the release of sensory neurotransmitters in response to activation of sensory nerve terminals produced by various stimuli such as an increase in extracellular K^+ concentration, a decrease in tissue pH or electrical stimulation (Szolcsanyi, 1996; Szolcsanyi et al., 1998). Regarding the neurotransmitters involved, most evidence favours an important role for sensory neuropeptides such as calcitonin gene-related peptide (CGRP), substance P, neurokinin A, somatostatin and possibly nitric oxide (NO) as an atypical neurotransmitter (Nijkamp and Folkerts, 1995; Szolcsanyi, 1996). We therefore postulated that the cisplatin-induced reduction in bronchoconstrictory responses to field stimulation was associated with a decrease in bronchial sensory neuropeptide release.

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2. Materials and methods

2.1. Ethics

The experiments performed in the present work conform to European Community guiding principles for the care and use of laboratory animals. The experimental protocol applied has been approved by the local ethical boards of the Universities of Pecs and Debrecen, Hungary.

2.2. Experimental groups and animals

The study was carried out with 20 Wistar male rats weighing 270–320 g. They were housed in an animal room 12-h light and dark periods a day, temperature of 22–25 °C, humidity of 50–70% with five animals per pen fed commercial laboratory chow and tap water ad libitum. The animals were randomized 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 5 days; Cisplatin-treated: animals treated with 1.5 mg/kg cisplatin (Bardos et al., 2003) with 75 mg/kg mannitol i.p. once a day over 5 days. Body weight was measured each day over the experimental period.

2.3. Isometric tension measurements

Isolated segments of the main bronchi (2 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, Hungary) containing Krebs solution (in mM): NaCl 119, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.5, KCl 4.7, $CaCl_2$ 2.5, glucose 11). The organ fluid was gassed with 95% O_2 and 5% CO_2 to maintain pH at 7.2±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 12 mN). Eight rings were prepared from eight animals in each group. To investigate 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, after the studies on neural effects on mechanical activity had been accomplished.

2.4. Neurotransmitter release studies

These have been described in detail elsewhere (Nemeth et al., 1999b). In brief, following exsanguinations, the lower third of the tracheae with the main bronchi were removed, cleaned of fat and adhering connective tissues. They were prepared for perfusion in a temperature (37 °C) and pH (7.2) controlled oxygenized Krebs solution over 60 min. Electrical field stimulation (20 V, 0.1 ms, 20 Hz, 100 stimuli) was applied to elicit neurotransmitter release. Calcitonin gene-related peptide (CGRP), substance P, and somatostatin concentrations were determined from 200 μl samples of organ fluid of the preparations by means of radioimmunoassay methods developed in our laboratories as described previously (Nemeth et al., 1999b). Determinations were done prior to as well as immediately and 2 min after field stimulation.

2.5. Nerve conduction velocity studies

This series of experiments was carried out to verify/exclude sensory neuropathy involving unmyelinated slow conducting 'C' fibres shown to play an important role in bronchomotility. Left saphenous nerve conduction velocity was determined in animals from both groups as described (Nemeth et al., 1999a,b; Szilvassy et al., 2000). In brief, in artificially ventilated animals anaesthetized with sodium pentobarbital (80 mg/kg i.p.) the nerve was prepared, cleaned of fat and 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 compound 'A' and 'C' signals were determined for calculation of average conduction velocity. The inter-electrode distance was divided by the interval between the end of the stimulatory impulse and the appearance of the corresponding 'A' and 'C' signals (Janig and Lisney, 1989). The cisplatin-induced 'C' signal delay was used for characterization of cisplatin-induced sensory neuropathy.

2.6. Study design

The animals in either group were anaesthetized for femoral nerve conduction velocity studies 11 days after the last cisplatin/vehicle dose. After completion of these studies, the tracheae with the main bronchi were removed. Two-millimeter long segments from the main bronchi were used for isometric tension measurements, whereas the rest of the tissues were utilized for neuropeptide release studies. Schematic representation of the experimental protocol is seen in Fig. 1.

2.7. Drugs and chemicals

All drugs and chemicals used in this study were purchased from Sigma (St. Louis, USA), except cisplatin and its solvent, which were obtained from TEVA-BIOGAL (Debrecen, Hungary) and chemicals used for radioimmunoassay determinations, which were from sour-

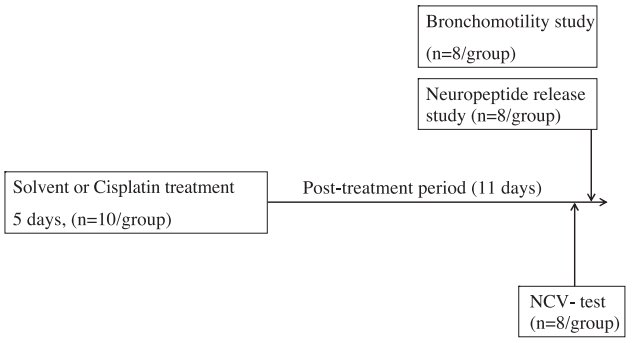

Fig. 1. Schematic representation of the experimental protocol applied. The animals randomized into two groups received either cisplatin (1.5 mg/kg/day i.p.) or its vehicle over 5 days. This period was followed by 11-day interval. The rats were then checked for the development of sensory neuropathy by means of femoral nerve conduction velocity studies. The animals were then sacrificed for in vitro bronchomotility investigations and bronchial neuropeptide release studies.

ces as follows: [Tyr^1]-somatostatin-14 and Tyr-α-CGRP-(23–37) were purchased from Bachem (Bubendorf, Switzerland). The Subtance P RIA tracer was from Amersham (Little Chalfont, UK); Tween-80, NaH_2PO_4, Na_2HPO_4, NaCl and CH_2Cl_4 from Reanal (Budapest, Hungary); trifluoroacetic acid (TFA) and piperine from Fluka (Buchs, Switzerland); high pressure liquid chromatography (HPLC)-grade methanol from Carlo Erba (Rodano, Italy); Substance P antiserum was provided by Prof. G.J. Dockray, University of Liverpool, SOM and CGRP antiserum by Dr. T. Görcs, University Medical School of Budapest. Polypropylene RIA tubes (12×75 mm) were obtained from Merck (Darmstadt, Germany). ^{125}I-labelled Tyr-α-CGRP-(23–37) and ^{125}I-labelled [Tyr^1]-somatostatin-14 were prepared in our laboratory (Németh et al., 2002).

2.8. Statistical analysis

The isometric tension and nerve conduction velocity data expressed as means±standard deviation (S.D.) were evaluated with analysis of variance (ANOVA) followed by a modified *t*-test according to Bonferroni's method (Wallenstein et al., 1980). The blood chemistry data and sensory neuropeptide levels were evaluated by Student's *t*-test for unpaired data.

3. Results

3.1. Exclusions

Two cisplatin-treated animals had to be excluded from the experiments, one of them died, and the other did not show any evidence for the development of sensory neuropathy in response to the cisplatin treatment schedule applied.

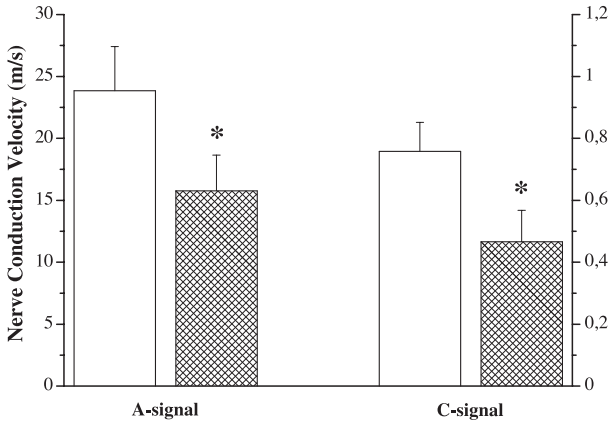

Fig. 2. Cisplatin-induced decrease in fast conducting myelinated (left ordinate) and slow conducting unmyelinated (right ordinate) fibres of the femoral nerve. Measurements were done 11 days after a series of five intraperitoneal injections of cisplatin (1.5 mg/kg/day) and/or its vehicle. The data are means±S.D. obtained from eight animals per group. *Significantly different from control at $P \leq 0.05$.

3.2. Body weight and rectal temperature

Body weight decreased from pre-treatment value of 294±22 to 231±19 g ($P<0.05$) in the group of cisplatin-treated animals. The rats treated with the solvent for cisplatin exhibited a tendency to weight gain (306±20 vs. pre-treatment 289±23 g). Rectal temperature did not change during the experimental period in either group.

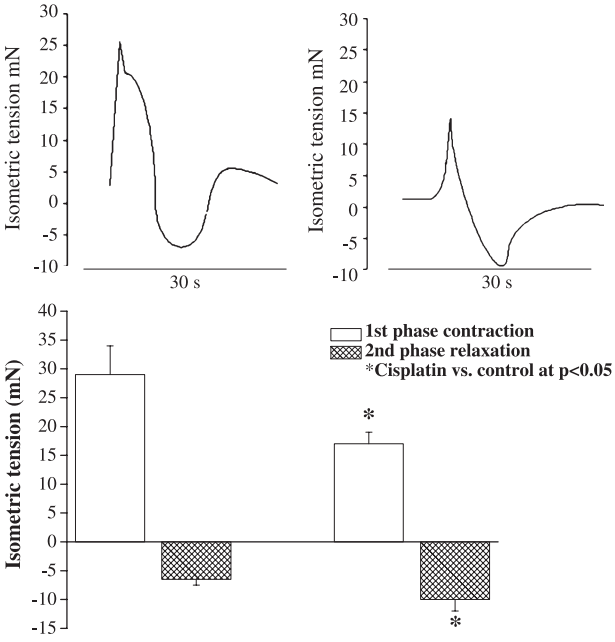

Fig. 3. Original tracings (top) and summarizing data (bottom) representing changes in isometric tension in bronchial preparations from control (left tracing and columns) and cisplatin-treated (right) animals in response to electrical field stimulation. Note the decrease in contractions in preparations from the cisplatin-treated animals. The data are means±S.D. obtained from eight values per group. *Significantly different from control at $P \leq 0.05$.

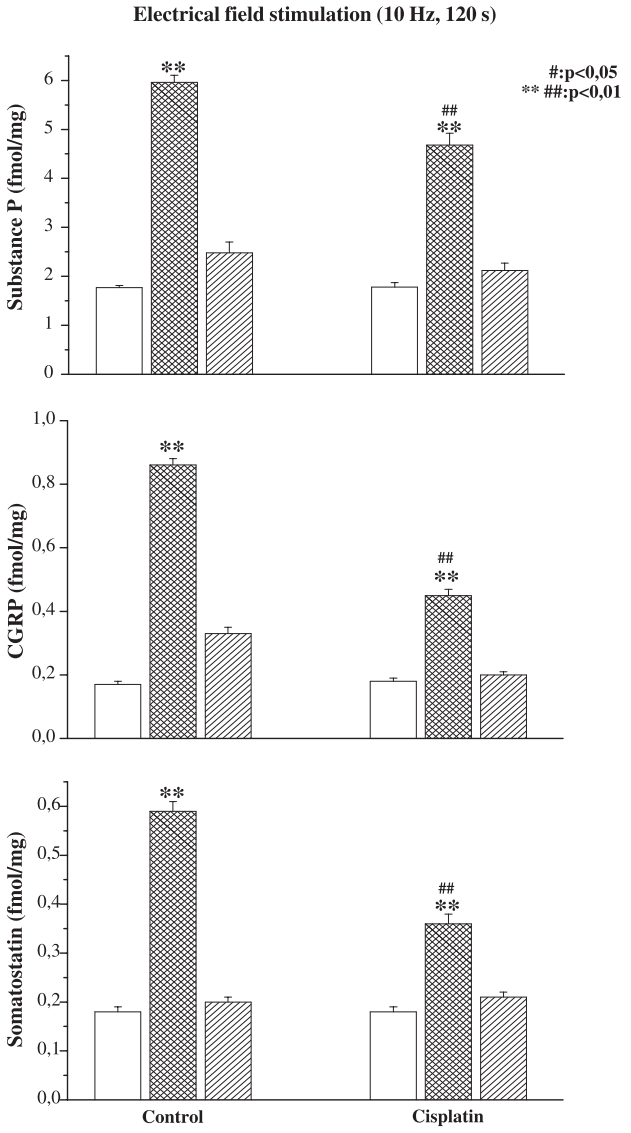

Fig. 4. Effect of field stimulation on sensory neuropeptide release from bronchial preparations from normal (vehicle-treated), and cisplatin-treated rats. Determinations were done prior to (open bars) as well as immediately (cross-hatched bars) and 2 min after (hatched bars) field stimulation. The data are means±S.D. obtained from eight preparations per group. *Significantly different from corresponding 'normal' values at $P<0.05$.

3.3. Nerve conduction velocity

Fig. 2 shows cisplatin-induced decrease in nerve conduction velocity in fast conducting myelinated (A fibres) and slow conducting unmyelinated (C) fibres. At a stimulation intensity suprathreshold for A- (0.5 V, 5 Hz) or C- (3 V, 5 Hz) fibres, nerve conduction velocity significantly decreased in rats with preceding treatment with cisplatin compared to those treated with the solvent for cisplatin.

3.4. Changes in isometric tension

Preparations from normal animals exhibited a biphasic response to electrical field stimulation i.e. an initial contraction was followed by relaxation (Fig. 3). The rings from cisplatin-treated rats responded with attenuated contractions to field stimulation compared to those from solvent-treated animals (Fig. 3). Nevertheless, the relaxation response to the field-stimulation protocol applied was of higher amplitude and shorter duration in rings from the cisplatin-treated animals than in controls. Field stimulation failed to induce any change in tension in rings pre-incubated with tetrodotoxin (data not shown).

3.5. Sensory neuropeptide release

It is seen from data in Fig. 4 that the field stimulation-induced increase in sensory neuropeptide release was significantly attenuated in preparations from animals with sensory neuropathy induced by cisplatin.

4. Discussion

These results confirm previous findings obtained in guinea-pigs (Szilvassy et al., 2000) that a 5-day treatment with cisplatin attenuates field stimulation-induced bronchoconstriction in vitro in preparations from rats. This attenuated bronchomotor response occurs in parallel with a significant decrease in the release of sensory neuropeptides known to play a role in neurogenic regulation of bronchomotility such as that of somatostatin, substance P and CGRP in response to a highly standardized field stimulation challenge. This parallelism is the major original finding of the paper. The field stimulation-induced bronchomotor response and the neuropeptide release were blocked by tetrodotoxin, a fast Na^+ channel blocker, thus, both can be considered to be of neural origin. These decreased responses were accompanied by a decline of femoral nerve conduction velocity in the cisplatin-treated animals. Since the nerve conduction velocity test is widely accepted as the 'gold standard' of peripheral neuropathy (Kato et al., 1998; Cameron and Cotter, 1997; Love et al., 1996), it is also confirmed that the cisplatin-treated animals suffered from sensory neuropathy 11 days after the 5-day treatment period.

Bronchial tissue is densely innervated by unmyelinated sensory fibres containing substance P, CGRP, somatostatin and neurokinin A (Lundberg et al., 1983, 1984). These fibres originate from the vagus nerve with cell bodies in jugular, nodose and dorsal root ganglia (Springall et al., 1987). As far as the regulatory role of these fibres in bronchomotility is concerned, it is closely linked to the so-called sensory effector function of these fibres. The essence of this particular function is that these fibres release their neurotransmitters into adjacent areas subsequent to activation attained by various stimuli such as an increase in extracellular K^+ concentration, decrease in pH (tissue acidosis) or electrical stimulation either with or without involvement of local reflexes (Szolcsanyi,

1996; Szolcsanyi et al., 1998; Németh et al., 2003). The neurotransmitters, once released, produce various responses, for example, in case of CGRP and substance P, changes in vascular tone and permeability and/or bronchoconstriction (Lundberg et al., 1983, 1984). As a methodological approach, these sensory nerve terminals locate in bronchial mucosa superficially enough to release neurotransmitters in response to electrical field stimulation at parameters selective for neural elements in sufficient quantities both to be detectable by analytical methods and to induce marked, predominantly NANC bronchoconstrictory responses. This enabled our experimental paradigm of studying neurogenic bronchomotiliy in relation to sensory neuropeptide release in cisplatin neuropathy in vitro. Since sensory neuropeptides play a major modulatory role in NANC bronchial motility, previous studies anticipated that a decreased availability of excitatory neuropeptides to be released by field stimulation were responsible for the feeble NANC contractile responses in bronchial preparations from cisplatin-treated animals similar to sensory neuropathy associated with advanced diabetes (Nemeth et al., 1999b; Szilvassy et al., 2002).

Of course, the current work can not be aimed to overemphasize the role of sensory neuropeptides in bronchomotility by that the experiments were focused on neuropeptide release. Our previous work revealed that it was the slow component of the bronchial contractile response to electrical field stimulation (shown to be capsaicin-sensitive), which disappeared in cisplatin neuropathy, whereas only a moderate decrease in the fast component (shown to be atropine- sensitive) was seen in preparations from such animals (Szilvassy et al., 2000). There is no doubt that acetylcholine is a neurotransmitter of crucial importance in bronchoconstrictive responses to electrical stimulation in both rats and several other species. As shown in Fig. 3, some of the rapid component of the bronchial contractile pattern is preserved in our cisplatin neuropathy model, which suggests that cholinergic pathways are also connected to some degree. The contractile pattern in Fig. 3 discloses the complete lack of the slow component of the field stimulation-induced contraction in preparations from cisplatin-treated animals, which is in good correlation with the significant decrease in the release of the three sensory neuropeptides measured.

The cisplatin treatment schedule applied yielded characteristic features of sensory neuropathy in that a significant decrease in femoral nerve conduction velocity occurred in both fast and slow conducting fibres. This is in good accordance with our previous results in which a similar subacute treatment with high dose cisplatin induced peripheral neuropathy in guinea pigs (Szilvassy et al., 2000). Regarding the mechanism underlying cisplatin-induced peripheral neuropathy, it has been found that the primary targets for cisplatin neurotoxicity are the dorsal root ganglia cells (Gispen et al., 1992; Barajon et al., 1996). In these cells, cisplatin induces an accumulation of sensory neuropeptides, suggesting an impaired axonal transport of sensory neurotransmitters to peripheral axon terminals (Barajon et al., 1996). Since the effector function of bronchial sensory nerves is underpinned by sensory neuropeptide release, it is not surprising that cisplatin-induced peripheral sensory neuropathy is reflected in impaired neurogenic bronchoconstriction as a consequence of a decrease in peripheral neuropeptide availability.

Theoretically, another possibility is that the enhanced relaxation response of preparations from cisplatin-treated animals to field stimulation may mask the entire contractile response showing contractions smaller than those seen in control. As shown in one of our previous papers, the exaggerated tracheal relaxation response associated with cisplatin neuropathy is underlain by an increased superoxide production (Szilvassy et al., 2000), at least in preparations from guinea pigs. Since this relaxation could completely be blocked by pharmacological inhibition of NO synthesis, it was concluded that this might have been related to an increase in peroxinitrite production, which otherwise is characteristic of cisplatin-induced neuropathy (Matsushima et al., 1998).

To the best of our knowledge, this work is the first to describe that the attenuated bronchomotor response in cisplatin-induced sensory neuropathy is related to a decrease in sensory neuropeptide release. Since non-adrenergic, non-cholinergic contractile agents such as substance P and CGRP together with tachykinins play an important role in neurogenic bronchoconstriction (Lundberg and Lou, 1996; Szolcsanyi, 1996), this means that bronchi of animals suffering from peripheral neuropathy characterized by a decreased availability of sensory neuropeptides are less prone to contract in response to neural and antigen challenges. Besides diabetes (Szilvassy et al., 2002), cisplatin-induced neuropathy is another example for this phenomenon. Beyond providing some approach as to why bronchial hyper-reactivity is attenuated in diabetes and drug-induced peripheral sensory neuropathy, the results also call attention to pharmacological exploitation of the sensory neuropeptide release/effect-bronchial smooth muscle contraction pathway to confer protection on patients at risk of bronchial hyper-reactivity.

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