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Short communication

Cisplatin acutely reduces 5-hydroxytryptamine-induced vagal depolarization in the rat: protective action of dexamethasone

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Abstract

The effect of the anti-cancer cytotoxic drug cisplatin on KCl and 5-hydroxytryptamine (5-HT)-induced depolarization in the rat isolated cervical vagus nerve was investigated using the 'grease gap' extracellular recording technique. KCl (10 mM) perfused onto the isolated nerve previously incubated for 2 h in 10 μM cisplatin initiated a d.c. potential of 1.06 ± 0.09 mV compared to a potential of 1.29 ± 0.13 mV in control nerves. Perfusion with 5 μM 5-HT produced a markedly reduced depolarization (0.23 ± 0.02 mV) in cisplatin-treated nerves compared with control nerves (0.42 ± 0.04 mV, $P = 0.005$). This effect was enhanced when 5-HT was reapplied 30 min later (0.19 ± 0.02 mV in cisplatin-treated compared with 0.42 ± 0.03 mV in controls, $P < 0.0001$). The inhibitory effect of cisplatin on 5-HT-induced depolarization was found to be significantly ($P = 0.004$) reduced by the addition of dexamethasone (10 μM) to the incubation buffer (0.34 ± 0.04 mV). These results are discussed in the light of the emetic and neurotoxic effects of cisplatin and the protective effects of dexamethasone.

Keywords: Vagal depolarization; Vagus nerve; 5-HT (5-hydroxytryptamine, serotonin); Cisplatin; Dexamethasone; (Rat); (Grease gap)

1. Introduction

Cisplatin is a cytotoxic drug used in the treatment of malignant tumours. Side-effects of acute cisplatin administration include nausea and vomiting (Andrews and Davis, 1993 for review). The mechanism involves 5-hydroxytryptamine (5-HT) release from enterochromaffin cells in the mucosa of the upper gastrointestinal tract, activating 5-HT_3 receptors on vagal afferents projecting to the brain stem to initiate the vomiting reflex (Leslie and Reynolds, 1993). Acutely cisplatin also appears to directly influence neurones as Scott et al. (1994) showed a concentration-related (0.1–10 μM) increase in excitability of cultured rat dorsal root ganglion (DRG) cells. In view of this observation the present study investigated the potential interaction between cisplatin and 5-HT on the cervical vagus nerve in the rat using the 'grease gap' preparation. Furthermore, as dexamethasone has been shown to attenuate the emetic response of patients undergoing cisplatin treatment (Aapro and Alberts, 1981) by an unidentified mechanism, experiments were also carried out to determine whether the presence of dexamethasone altered the vagal response to cisplatin and 5-HT.

2. Materials and methods

Male Wistar rats (200–220 g; Biological Research Facilities, SGHMS) were used throughout this study. The animals were killed by stunning and cervical dislocation. Sections (~ 2 cm) of both left and right cervical vagus nerves caudal to the nodose ganglion were rapidly removed and placed in Krebs-Henseleit buffer (see below) with or without cisplatin, transplatin or dexamethasone (see below), continually gassed with 95% O_2/5% CO_2 and kept on ice for 2 h. The connective tissue sheath surrounding each nerve was then carefully removed under a dissecting microscope and the desheathed nerve placed across two compartments of a three-compartment Perspex bath so that 50% of the nerve lay in one compartment whilst the other half

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projected through a greased (Dow Corning high vacuum grease) slot into the other compartment. The third compartment was redundant. The d.c. potential across the two compartments was measured with silver/silver chloride electrodes that were secured in the cut-off end of 1 ml plastic syringes that inserted into the base of each compartment. The nerve was superfused from an elevated reservoir of gassed buffer at a rate of ~2 ml/min and temperature of 26° C through the central compartment only. The buffer in the other compartments was also maintained at 26° C and changed before each test drug was applied. The signals were amplified and filtered by a Maclab 4e (AD Instruments) system and recorded using the accompanying Chart software.

2.1. Experimental protocols

As indicated above, the nerves were mounted in the baths 2 h after removal from the animal, one nerve having been kept in gassed Krebs-Henseleit containing either cisplatin (10 μM), dexamethasone (10 μM), cisplatin plus dexamethasone, or transplatin (10 μM), whilst the other nerve was placed in the corresponding control solution, e.g. the control for dexamethasone in some cases was vehicle, whereas the control for dexamethasone plus cisplatin was cisplatin alone in some instances and dexamethasone alone in other experiments. Cisplatin and transplatin were also tested against vehicle. The data from all experiments were then pooled and this accounts for the unequal numbers in each group. The concentrations of cis- and transplatin used were the same as those in the dorsal root ganglion study by Scott et al. (1994) and in the case of cisplatin is in the range reported to occur in the tissue of patients undergoing therapy. The nerves were then left in the baths for a further 30 min for the recordings to stabilise before potassium chloride (KCl; 10 mM) in Krebs-Henseleit ± cisplatin, transplatin or dexamethasone was perfused on to the nerve for 2 min, in order to provide a 'test' depolarization. After a further 30 min, 5-hydroxytryptamine (5-HT; 5 μM) was administered for 2 min. A second dose of 5-HT was administered 30 min later. The concentration of 5 μM 5-HT was used as this has been shown to be submaximal (Ireland and Tyers, 1987; Tresize et al., 1993; Bley et al., 1994) and we have previously confirmed this (Woods and Andrews, unpublished data). A submaximal concentration of 5-HT would allow both inhibitory and stimulatory effects of cisplatin to be demonstrated.

The values reported for depolarisation during each treatment are the change in d.c. potential from the baseline to the peak of the response during KCl or 5-HT application. Values are expressed as mean ± S.E.M., n = number of nerves from different animals. Statistical significance was tested using one-way analysis of variance and post-hoc Scheffe's test and independent Student's t-tests.

2.2. Drugs

Cisplatin (cis-platinum II diammine dichloride), transplatin (trans-platinum II diammine dichloride), dexamethasone (dexamethasone 21-phosphate, disodium salt) and 5-hydroxytryptamine (hydrochloride) were all obtained from Sigma (Poole, Dorset, UK) and dissolved in Krebs-Henseleit buffer (NaCl 120.8 mM, KCl 4.7 mM, KH_2PO_4 1.2 mM, $MgSO_4 \cdot 7H_2O$ 1.2 mM, $NaHCO_3$ 24.9 mM, $CaCl_2$ 2.4 mM, glucose 5.6 mM). Stock solutions of both cisplatin and transplatin were dissolved in Krebs-Henseleit buffer at 80° C in a water bath to ensure complete dissolution.

3. Results

Cisplatin, transplatin and dexamethasone had no acute effect on the d.c. potential across the vagus nerve section in this preparation, i.e. none of these compounds induced depolarization.

The mean values of all KCl-induced depolarizations in all experimental groups are shown in Fig. 1. There were no significant differences between any of the treated (cisplatin, transplatin, dexamethasone) groups and the control (untreated) nerves.

Fig. 2 shows the 5-HT-induced depolarizations in the nerves treated with cisplatin, dexamethasone, or transplatin in various combinations. Cisplatin (10 μM) caused a significant ($P = 0.005$) reduction in the magnitude of the 5-HT-induced depolarization compared to controls. This attenuation of the response was markedly ($P = 0.004$) reduced by dexamethasone (10 μM; 0.32 ± 0.04 and 0.30 ± 0.03 mV) whereas dexa-

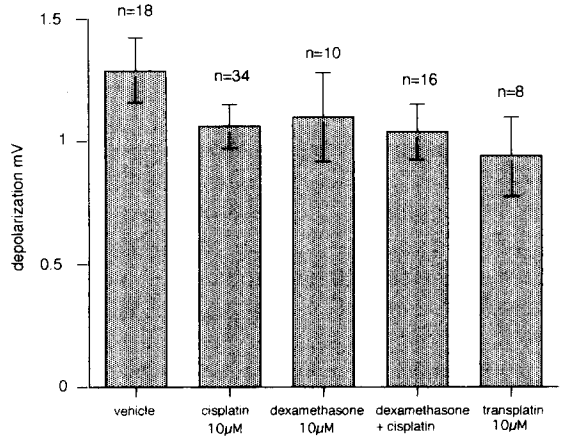

Fig. 1. Effect of KCl on the d.c. potential (mean ± S.E.M.) across the isolated rat vagus. There were no significant differences between the groups.

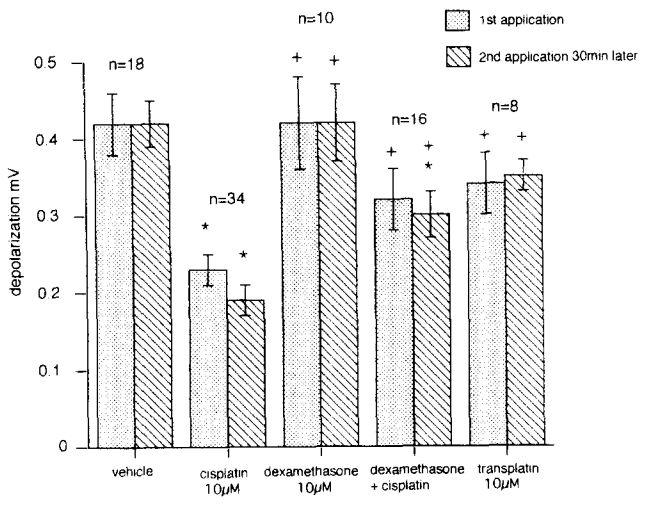

Fig. 2. Effect of 5-hydroxytryptamine on the d.c. potential (mean ± S.E.M.) across the isolated rat vagus. * Significant difference from vehicle-treated nerves, $P < 0.01$; + significant difference from cisplatin-treated nerves, $P < 0.01$.

methasone alone had no effect on the response to 5-HT (0.42 ± 0.06 and 0.42 ± 0.05 mV).

Nerves incubated in transplatin (10 μM) had 5-HT-induced depolarizations that, although smaller than those of the control group (0.34 ± 0.04 and 0.35 ± 0.02 mV), were not significantly different from controls but were significantly different from cisplatin-treated nerves ($P < 0.05$).

4. Discussion

These studies demonstrate that cisplatin but not transplatin reduced the depolarising response to 5-HT but not KCl in the rat isolated cervical vagus nerve. This depolarization is mediated by 5-HT_3 (Ireland and Tyers, 1987; Newberry et al., 1993; Bley et al., 1994) and 5-HT_4 receptors (Rhodes et al., 1992; Bley et al., 1994), with the 5-HT_3 component being predominant, especially at temperatures below 31° C (Bley et al., 1994). Cisplatin has been previously demonstrated to be neuroactive; however, in contrast to the present study, Scott et al. (1994) showed that cisplatin decreased input conductance of dorsal root ganglion cells and increased excitability as reflected by the lowered action potential threshold when administered acutely. However, chronically treated DRG cells were less excitable than control cells with an increased threshold for action potentials. Although we have failed to demonstrate acute effects of cisplatin alone on the rat isolated vagus, we have shown an effect which appears to correlate more closely with the decreased excitability of DRG cells shown by Scott et al. (1994). The apparent difference in the acute effects of cisplatin between the DRG and the vagus nerve could reflect differences between the response of axons and neurone cell bodies to cisplatin which may in turn be a reflection of membrane characteristics and cellular biochemistry. The decreased depolarization response to 5-HT in the cisplatin-treated vagus may reflect a degree of axonal damage although as the response to KCl was not significantly affected, this could not account for the entire effect. It appears more likely that cisplatin has its effect by a more selective action on the vagal 5-HT receptors. This action of cisplatin appears to be stereoselective as transplatin (10 μM) did not have a significant effect on 5-HT-induced depolarization. This is mirrored by the steric interaction involved in the anti-tumour activity (Connors et al., 1972) and the neurotoxicity (Blisard et al., 1992) of cisplatin.

The relationship between the results from the present study and the mechanism(s) by which cisplatin induces emesis is not clear. It might have been expected that cisplatin would have enhanced the response to 5-HT providing a mechanism by which the the vagal response to 5-HT released from the enterochromaffin cells by cisplatin could be amplified. As the vagal axons in the present study lack a terminal generator region, the region of a sensory nerve most sensitive to pharmacological stimuli (Paintal, 1964), it is unlikely that this preparation would have revealed such an effect. In the aneasthetised ferret Andrews and Davidson (1989) failed to demonstrate direct excitation of single vagal afferent fibres by systemic cisplatin and in a more recent study of systemic cisplatin in the rat (Hillsley and Grundy, 1994) recorded an increase in mesenteric afferent discharge in 5 out of 11 experiments, a decrease in 2 and no effect in 4 experiments. The issue of whether cisplatin has direct effects on afferents in vivo is therefore unresolved and it is likely that the effects seen in the present study relate more to the neurotoxic effects of cisplatin rather than its emetic effects.

Dexamethasone reduced the effectiveness of cisplatin at decreasing the magnitude of 5-HT-induced vagal depolarization response most probably via a membrane-linked event. However, the mechanism is unknown. Several suggestions have been made about the mechanism of the anti-emetic effect of dexamethasone and related steroids (e.g. methylprednisolone) including the inhibition of a prostaglandin pathway (Rich et al., 1980), an effect on capillary permeability in the chemoreceptor trigger zone (Livera et al., 1985) and enhanced endorphin release (Harris, 1982). None of these proposed mechanisms is applicable to the protective effect of dexamethasone against cisplatin in the rat isolated vagus. In conclusion the results from this study provide a preliminary indication that cisplatin and dexamethasone may modulate vagal 5-HT receptors.

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