α-Melanocyte-Stimulating Hormone Has Protective Properties against the Toxic Effect of Cisplatin on Cultured Dorsal Root Ganglia

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INTRODUCTION

Cisplatin (CPt) is a powerful cytostatic and an important drug in the treatment of malignancies of the genitourinary tract, especially ovarian cancer. Like most cytostatics, it has a range of side effects, varying from nausea to neurotoxic effects. Renal toxicity, due to reduced glomerular filtration, can be overcome by diuretics and excessive water intake. Neurotoxicity, which occurs at high cumulative doses and is confined to the peripheral nervous system, is considered the main dose-limiting side effect. The result of the as yet unknown interaction of the drug with the peripheral nervous system is a distal, sensory neuropathy, characterized by tingling sensations and numbness in hand and feet, loss of sense of vibration, and a reduced propriocepsis. This neuropathy is progressive and may lead to a severe sensory ataxia. Remarkably, the motor system is not affected, nor is muscle strength diminished. Apparently, the drug affects the sensory ganglia but not the motor neurones, which points to a protective effect of the blood-brain barrier. The dorsal root ganglia (DRG), which contain the sensory neurones, are outside the blood-brain barrier. Indeed, it has been shown that CPt concentrations within the central nervous system are ten times lower than, e.g., in the DRG.[1] It is not known how CPt affects the sensory neurones, or indeed, whether it affects neurones, glia (Schwann or satellite cells), or both. There are indications that microtubule polymerization is affected,[2] which could lead to impaired neuronal functioning. A possible indication that glial cells are involved is that CPt-DNA adducts are only found in satellite cells in DRG, not in the neurones.[3] This finding led us to treat

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cultured Schwann cells with CPt and measure laminin production.[4] However, we were not able to find a protective effect of the melanocortin on Schwann cells. In this report we describe the effect of CPt on sensory neurones in chicken DRG in vitro, and protective effects of another melanocortin, α-melanocyte-stimulating hormone (α-MSH).

METHODS AND RESULTS

DRG were dissected from ED12 chick fetuses and cultured in a semisolid medium as described before.[6] They were treated with CPt (0–20 μg/ml), and

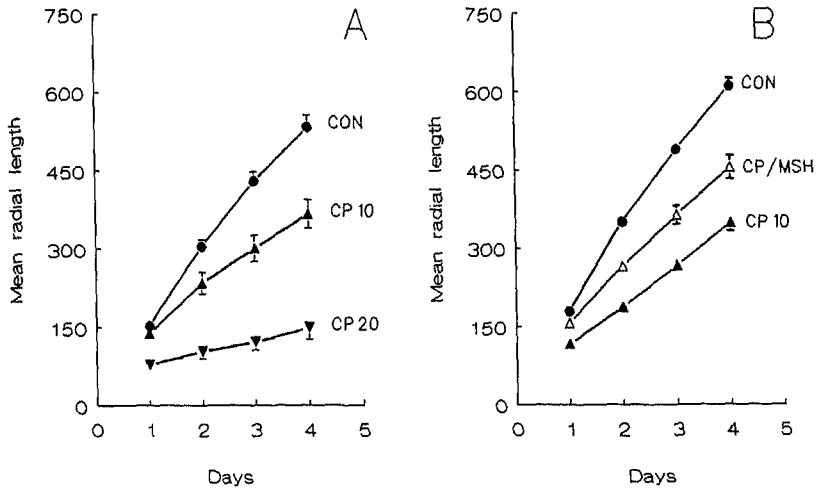

FIGURE 1. The outgrowth of neurites from chicken DRG in culture, given as mean radial length of the neurites. **A:** The effect of different concentrations cisplatin (CP): "CP10" means 10 μg cisplatin per ml and "CP20" means 20 μg/ml. CON represents the outgrowth of untreated, control DRG. The data are the average of 6–8 ganglia per treatment. Bars indicate the standard error of the mean (SEM). **B:** The effect of α-MSH on cisplatin-induced growth inhibition. α-MSH was capable of partially restoring outgrowth from DRG that had been treated with cisplatin (10 μg/ml). Data are the average of 6–9 ganglia. Bars indicate the SEM.

outgrowth was monitored daily by measuring the length of growing neurites (l_{av}). From these data the growth rate (r_g) was calculated. The average r_g in untreated cultures was 150 μm/day. CPt treatment reduced outgrowth from DRG in a dose-dependent way (FIGURE 1A). We choose 10 μg/ml CPt (= 33 μM) as the concentration at which outgrowth was inhibited by appr. 50% and treated the cultures with 10 nM α-MSH, together with CPt. In these cultures, a significant improvement of axonal outgrowth could be observed: the initial r_g was 53% higher and the l_{av} at day 4 was 455 μm (FIGURE 1B). At this concentration of α-MSH, no full recovery of outgrowth was obtained, but other concentrations may be capable of restoring the capacity fully.

DISCUSSION

These experiments show clearly that CPt is neurotoxic to DRG *in vitro* in a dose-dependent way and that 10 nM α-MSH can partially prevent this toxic effect, measured by the outgrowth of neurites from sensory neurones. This effect of α-MSH may be related to that of another ACTH-related peptide, Org2766, that has been proven effective in reducing sensory disturbances in patients treated with CPt.[4] This study, however, does not solve the question of which cell type CPt acts on. In view of the close trophic relation between satellite cells and sensory neurones, it may very well be that, e.g., CPt acts on the satellite cell,[5] leading to a diminished trophic support from this cell to neurones. α-MSH may restore the trophic-dystrophic balance in neurones by acting directly on neurones, or indirectly by stimulating glial cells. More studies are needed with dissociated cells and cocultures of different cell types to unravel which cell is the target for the trophic activity of melanocortins. Studies with dissociated spinal cord cells indicate that, in the absence of glial cells, neurones can respond to melanocortins.[7,8] Earlier we have detected a response of the myelin-associated enzyme CNPase in Schwann cells to α-MSH treatment.[9] Thus, it may be that Schwann or satellite cells do respond to melanocortins by producing, e.g., adhesion factors (laminin), growth factors, or their receptors, as is shown in peripheral nerve damage.[10] In conclusion, this study has shown that α-MSH has a neuroprotective effect *in vitro* which can be easily quantified. More experiments are needed to elucidate which cell type responds.

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