Perilymphatic application of $\alpha$–melanocyte stimulating hormone ameliorates hearing loss caused by systemic administration of cisplatin
Summary

It has previously been demonstrated that ototoxicity induced by systemic administration of cisplatin is reduced by concomitant systemic administration of α–melanocyte stimulating hormone (α–MSH). In this study we investigated the effects of cochlear, perilymphatic application of α–MSH during intraperitoneal administration of cisplatin. Guinea pigs, implanted with a round window electrode, allowing daily monitoring of the compound action potential (CAP), and a mini-osmotic pump, pumping at a rate of 0.25 µl/h either physiological saline or α–MSH solution (0.02, 2, 20 µg/ml), were treated daily with a bolus injection of cisplatin (2 mg/kg) until the electrocochleogram showed an increase in CAP threshold of 40 dB at 8 kHz. Then, cisplatin treatment was stopped, but intracochlear perfusion of α–MSH or physiological saline was continued for 10 days to evaluate possible effects of α–MSH on the expected recovery. On day 10, the animals were sacrificed and the cochleas were fixed and processed for histological analysis. All groups required an average of 6 to 7 days of cisplatin to reach the criterion CAP threshold shift. Ten days after cessation of the cisplatin treatment, recovery of the CAP was observed in all groups and at all frequencies, although it was more pronounced at the low frequencies. With respect to recovery, small statistically significant differences were found between the saline and the α–MSH co-treated groups. Histological results showed significantly less outer hair cell (OHC) loss in the group co-treated with 2 µg/ml α–MSH as compared to the group co-treated with saline. Thus, since α–MSH was delivered directly into the cochlea, the ameliorating effect of α–MSH on OHC survival is exerted by means of a cochlear target and not through interaction with a systemic factor.
Cisplatin is a widely used antineoplastic drug, which is effective in the treatment of different types of epithelial tumors. However, in humans, chronic treatment with cisplatin may result in ototoxic side effects such as high-frequency hearing loss and tinnitus (Schweitzer, 1993). Animal studies have shown that chronic cisplatin administration leads to loss of outer hair cells (OHCs) in the cochlea, with the hair cells in the basal turn being more severely affected than those in the middle and apical turns (Tange, 1984; Hoeve et al., 1988; Saito and Aran, 1994; Cardinaal et al., 2000a). The ototoxic effects of cisplatin are not limited to the auditory hair cells; the stria vascularis (Tange and Vuzevski, 1984; Kohn et al., 1988; Zheng and Gao, 1996; Kohn et al., 1997; Meech et al., 1998; Cardinaal et al., 2000a,b) and spiral ganglion cells (Cardinaal et al., 2000b; Hamers et al., 2003) are also affected. These ototoxic limitations can be (partially) overcome with the application of compounds that are known for their neuroprotective effects in cisplatin-induced sensory neuropathies. A family of compounds that have distinct neuroprotective properties are the melanocortins (Gispen, 1990). Previous work in our laboratory has shown that systemic administration of some of these melanocortins, e.g. the AdrenoCorticoTropic Hormone (ACTH)-analog ORG 2766 and the naturally occurring ACTH fragment $\alpha$-melanocyte stimulating hormone ($\alpha$-MSH), protect the cochlea from cisplatin-induced damage (Hamers et al., 1994; De Groot et al., 1997; Stengs et al., 1998; Heijmen et al., 1999; Smoorenburg et al., 1999; Cardinaal et al., 2000c; Wolters et al., 2003; Hamers et al., 2003). However, the systemic concomitant administration of ORG 2766 or $\alpha$-MSH resulted in a clear dichotomy; individual animals were either completely protected from cisplatin ototoxicity with normal auditory thresholds and no OHC loss or demonstrated increased thresholds similar to animals treated with cisplatin alone (Hamers et al., 1994; Stengs et al., 1998; Heijmen et al., 1999). Therefore, further quantitative characterization of the interaction between melanocortin effects and cisplatin-induced ototoxicity was needed. Introduction of a longitudinal animal model allowed us to monitor the cochlear sensitivity and the beneficial effects of $\alpha$-MSH and ORG 2766, via a permanent round window electrode, on a day-to-day basis (Hamers et al., 2003). Neither of the co-treatments affected the mean number of cisplatin injections necessary to reach the criterion threshold shift ($\geq 40$ dB at 8 kHz). Nevertheless, both $\alpha$-MSH and ORG 2766 enhanced recovery of CAP thresholds and CAP amplitudes at high sound pressure levels. Furthermore, hair cell loss was significantly lower in the melanocortin co-treated groups. On the one hand, the beneficial effects of $\alpha$-MSH and ORG 2766 could be evoked through a target within the inner ear. On
the other hand, systemically applied melanocortins may exert their effects indirectly, e.g. by accelerating renal clearance of cisplatin from the body, resulting in decreased amounts of cisplatin that can reach the cochlea. This latter explanation has been found to be the basic mechanism by which the sulphur-containing amino acid D-methionine acts as an otoprotective agent (Ekborn et al., 2002).

The present study was designed to investigate the issue of the primary target for α−MSH. A way to prevent the systemic effect of the protective compound is to apply the protective agent exclusively and directly to the cochlea. To accomplish this, a new longitudinal model was used, which involved administration of α−MSH directly into the scala tympani via a mini-osmotic pump system, while cisplatin was administered intraperitoneally. Daily recordings of the compound action potentials (CAPs) as a function of frequency and level were made from a permanent round window electrode. This study complements a similar study with a mirrored experimental design: cisplatin was applied directly into the scala tympani, whereas α−MSH was administered subcutaneously, in combination with permanent recordings (Wolters et al., 2003). In that study a clear ameliorating effect of α−MSH was found. It should be kept in mind, however, that positive results in this study would not completely rule out a systemic component, but positive results from the combination of the two studies would make that possibility extremely unlikely.

The experimental approach used in the present study has the additional advantage that since cisplatin is administered systemically, treatment can be terminated easily in contrast to an approach in which cisplatin is delivered directly into the cochlea through a mini-osmotic pump system. This allows for a better analysis of the role that α−MSH plays in recovery from cisplatin ototoxicity, especially since there are indications that α−MSH accelerates recovery (Hamers et al., 2003).

Materials and Methods

Animals and experimental design

Fifty-eight female albino guinea pigs (strain: Dunkin-Hartley, Harlan Laboratories, Horst, the Netherlands; weight 275-380 g) were implanted with a permanent round window electrode and equipped with a mini-osmotic pump containing sterile solutions of either α−MSH in saline or plain saline (0.9% NaCl). The pump fed these solutions directly into the scala tympani. The animals were housed, four together, in macrolon cages and had free access to food and water. They were maintained on a 12:12 h dark/light cycle. The ani-
mals were treated with a continuous perilymphatic perfusion of α-MSH or physiological saline, starting directly after surgery, and a daily injection of cisplatin, starting 3 days after surgery, until electrocochleograms showed ≥ 40 dB shift of the threshold at 8 kHz stimulation. When the criterion threshold shift was reached, cisplatin administration was stopped, but perilymphatic perfusion with α-MSH or physiological saline was continued for 10 days to evaluate possible effects of α-MSH on the expected recovery. During this period all animals were daily monitored by means of electrocochleography using the permanent round window electrode. On day 10, the animals were sacrificed and the ears were fixed and processed for histology. The animals were allotted at random to the different experimental groups. The care and use of the animals reported in this study were approved by the Animal Care and Use Committee of the University of Utrecht (DEC-UMC # 91035).

Preparation of pump, cisplatin and α-MSH solutions
A stock solution of cisplatin (1 mg/ml; Platosin®; Pharmachemie B.V., Haarlem, the Netherlands) was diluted with physiological saline to a final concentration of 0.1 mg/ml. It was administered intraperitoneally at a daily dose of 2.0 mg/kg body weight. The first injection was given 3 days after surgery. The dose was chosen based upon the previous experiments of Stengs et al. (1998) and Heijmen et al. (1999). The high dilution was chosen to stimulate diuresis and, thus, to minimize renal effects.
α-MSH (Bachem, Bubendorf, Switzerland) was dissolved in HCl (1 mM) containing 0.02% BSA (bovine serum albumin; BDH Chemicals Ltd., Poole, UK) at a concentration of 2 mg/ml (stock). This stock was further diluted with physiological saline containing 0.02% BSA to obtain the final concentrations used in this study (20, 2, and 0.02 µg/ml). The Alzet mini-osmotic pump (model #2004, Alza Corp., Palo Alto, CA, USA) was filled with either one of the three α-MSH solutions or 0.02% BSA containing saline and placed in a saline bath (37°C) for 40 hours or more before surgery, using a sterile container. After this, according to the manufacturers specifications, the pump worked immediately at a constant rate of 0.25 µl/h for 4 weeks.

Surgical techniques
Surgical implantation of the mini-osmotic pump and further techniques were performed according to the method described by Prieskorn and Miller (2000). The animals were anaesthetized with a mixture of ketamine and xylazine. The cannula (length: 7.4 cm) was filled with the same α-MSH solution as the pump. A mid-line incision was made on the dorsal surface of the head starting
2.5 cm anterior of bregma and continued post-auricularly to the base of the pinna. A superficial subcutaneous pocket was made in the back between the scapulae of the animal to accommodate the pump. Under sterile conditions the bulla of the left ear was opened retro-auricularly and the skull was exposed around bregma. A small hole was made at the base of the cochlea, approximately 0.5 mm below the round window. The cannula tip was placed into the hole until the silicone ball, 0.5 mm from the tip, was seated against the cochlea to prevent leakage. The round window electrode, made of insulated stainless-steel wire (diameter 0.175 mm including teflon insulation; Advent, Halesworth, UK) with an 0.5 mm diameter gold ball micro-welded (Unitek 80F; Unitek Equipment, Monrovia, CA, USA) to the exposed and flattened tip, was positioned on the round window and secured to the bulla with carboxylate cement (Durelon, ESPE dental supplies, Utrecht, The Netherlands). The wire was soldered to a Berg 22-26 gold terminal that fitted into a Berg 2x3 mini-latch housing (Farnell, Maarssen, The Netherlands). Stainless-steel screws were inserted through the skull and connected to the mini-latch housing via two silver wires connected to a gold terminal. The mini-latch housing was connected to the skull with dental acrylic cement, which also covered and insulated the stainless-steel screws and the electrodes. The pump was removed from the water-bath and attached to the cannula. Subsequently, the pump was placed in the subcutaneous pocket, the cannula was fixed to the skull, and the subcuticular layer was closed with a continuous vicryl suture; the skin was closed with vicryl using interrupted sutures.

**Electrocochleography**

Measurements were performed differentially with the round window electrode as the active electrode and two screws on the skull as reference and ground electrode, respectively.

Animals that did not have a normal threshold at 8 kHz (defined as a threshold at less than 25 dB SPL stimulus level) at the day after surgery or that showed a displaced cannula or signs of otitis media when sacrificed were excluded from further analysis. Trains of tone bursts of 2, 4, 8, and 16 kHz were used as stimuli. The tone bursts were constructed with cosine-shaped rise-and-fall times of 1 ms (1.5 ms at 2 kHz) and had a duration of 8 ms. The sound stimuli were produced in an open-field configuration with a Fame tweeter (Staffhorst Electronics, Utrecht, The Netherlands) positioned at 10 cm from the pinna. Consecutive tone bursts were presented with alternating polarity at 99 ms intervals in order to avoid synchronization with the mains frequency of 50 Hz. The responses were amplified (EG&G Instruments model 5113 amplifier, Te
Lintelo Systems, Zevenaar, The Netherlands), bandpass filtered between 1 Hz and 30 kHz, AD converted at 33 kHz, and stored on disk for off-line analysis. The animals were lightly restrained, but remained awake during all measurements, avoiding the disadvantages of repeatedly having to anaesthetize the animals. CAPs were obtained by adding the responses evoked by tone bursts of opposite polarity; Cochlear Microphonics (CM) by subtracting these responses. The CAP was measured relative to the positive summating potential (SP) and not relative to the baseline of the recording since, in principle, the CAP is superimposed upon the SP. The CM was measured as the peak-to-peak amplitude in the middle of the sinusoidal response. Electrocochleography was continued for 10 days following the cessation of cisplatin treatment.

ANOVA was performed on thresholds (3 µV iso-response levels) and amplitudes of CAPs and CM. Co-treatment was a between-subjects factor; time and stimulation frequency and level were within-subject factors. STATISTICA software was used.

**Histological techniques**

Ten days after the cessation of cisplatin administration the cochleas were fixed by intralabyrinthine perfusion with a tri-aldehyde fixative consisting of 3% glutaraldehyde, 2% formaldehyde, 1% acrolein, 2.5% dimethylsulfoxide in 0.1 M sodium cacodylate buffer (pH 7.4) followed by immersion in the same fixative for 3 h at room temperature. The cochleas were further processed according to the routine method for guinea pigs (De Groot et al., 1987). Semithin (1 µm) midmodiolar sections were cut and stained with 1% methylene blue and 1% azur II in 1% sodium tetraborate. In the midmodiolar sections the organ of Corti was examined at seven locations, separated by a half-turn spacing, and the number of OHCs present at each location was counted. OHC loss was expressed as the percentage of remaining OHCs per cross-sectioned half turn (2 transections each for the basal and middle turns; 3 transections for the apical turn), relative to the number of OHCs found in non-treated cochleas. All OHC counts were performed by two investigators, independently of one another, in a single-blind fashion (cf., De Groot et al., 1997; Cardinaal et al., 2000a; Wolters et al., 2003). ANOVA was used for statistical evaluation of the OHC counts. Co-treatment was a between-subjects factor and cochlear location a within-subject factor.
Results

General findings
Sixteen of the 58 operated animals showed an abnormal threshold at 8 kHz directly after surgery and fourteen animals demonstrated a displaced cannula or infections upon inspection of the left (operated) bulla when sacrificed. These animals were excluded from the experiment. Table 1 shows the distribution of the remaining animals (n=28) over the four experimental groups.

Table 1: Distribution of the animals over the experimental groups

<table>
<thead>
<tr>
<th>Pump filling</th>
<th>Number of animals</th>
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<tbody>
<tr>
<td>α−MSH (20 µg/ml)</td>
<td>6</td>
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<tr>
<td>α−MSH (2 µg/ml)</td>
<td>8</td>
</tr>
<tr>
<td>α−MSH (0.02 µg/ml)</td>
<td>8</td>
</tr>
<tr>
<td>Physiological saline</td>
<td>6</td>
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Effects of cisplatin on CAP thresholds in relation to co-treatment
During the first days of cisplatin treatment only small random changes in CAP threshold were observed. At some moment between day 5 and day 11 after the start of cisplatin administration, the animals showed a sudden deterioration of the CAP threshold. This was reported before when a similar experimental design was applied (Klis et al., 2000; Wolters et al., 2002; Hamers et al., 2003). Figure 1 shows the time required to reach the criterion threshold shift of ≥ 40 dB at 8 kHz.

Saline co-treated animals reached this criterion threshold shift after an average of 6.8 days. Animals that received the lowest concentration of α−MSH (0.02 µg/ml in the pump) reached the criterion threshold shift almost one day earlier (6 days) and also showed significantly less variability (Levene’s test, P<0.026). Animals receiving higher concentrations of α−MSH (2 or 20 µg/ml in the pump) reached the criterion threshold shift after 7.3 and 6.7 days of cisplatin administration, respectively. ANOVA showed that the mean number of days of cisplatin administration necessary to cause hearing loss did not differ between the four groups in a statistically significant way (F(3,24)= 0.719, P=0.55).
Figure 1: The time required to induce a hearing loss of ≥ 40 dB in the 3 µV CAP iso-response level (threshold) at 8 kHz for the four different groups.

Figure 2: Maximum shift in CAP threshold (3 µV criterion) at the day cisplatin administration was terminated or shortly thereafter, as a function of stimulus frequency for the four different groups.
CAP thresholds

Maximum CAP threshold shifts occurred either at the day cisplatin administration was terminated or 1 to 2 days later. After the maximum CAP threshold shift was reached, the threshold recovered in 50% of the animals (cf., Fig. 3). Maximum threshold shift in dB as a function of frequency is shown in Figure 2 for all groups. Although the hearing loss occurred within a broad frequency range, it clearly increased as a function of frequency. ANOVA showed a significant main effect of frequency ($F_{(3,72)}=112.8$, $P<0.001$) but no main effect of co-treatment ($F_{(3,24)}=2.07$, $P=0.13$). Although only a small significant interaction between

Figure 3: Build-up and recovery of the CAP threshold shift at 2, 4, 8, and 16 kHz (± S.E.M.) as a function of time for the 4 different groups. The horizontal black bar represents the last 6 days of cisplatin administration. Day 0 represents the day that cisplatin administration was discontinued. CAP threshold baseline values between day -6 and day -2 before cessation of cisplatin administration were averaged for each individual animal and set to zero. These values were taken as the reference “normal hearing level” (nHL).
both factors \( (F(9,72)=1.97, P=0.055) \) was found, we performed an analysis of the effect of \( \alpha \)-MSH co-treatment for each frequency separately. Animals co-treated with 2 \( \mu \)g/ml \( \alpha \)-MSH showed a significantly smaller threshold shift than animals treated with saline at 2 and 4 kHz \( (F(1,12)=9.60, P=0.009 \) and \( F(1,12)=16.90, P=0.001, \) respectively), but not at 8 and 16 kHz \( (F(1,12)=0.98, P=0.343 \) and \( F(1,12)=0.185, P=0.67, \) respectively).

Figure 3 shows CAP thresholds at 2, 4, 8, and 16 kHz, based on the 3 \( \mu \)V response amplitude criterion as a function of time. Since baseline threshold values varied between animals, the CAP threshold baseline values between day -6 and day -2 before cessation of cisplatin administration were averaged for each individual animal and set to zero. These values were taken as the reference “normal hearing level” (nHL). As mentioned before, pronounced recovery of the CAP threshold was observed in all groups after cessation of cisplatin administration and it was observed at all frequencies, although it was more prominent at the lower frequencies. Statistical analysis (ANOVA for repeated measurements) of the data starting at day 0 showed a significant effect of time (recovery) at all frequencies (2 kHz: \( F(9,216)=44.13, P<0.001 \); 4 kHz: \( F(9,216)=20.67, P<0.001 \); 8 kHz: \( F(9,216)=10.88, P<0.001 \); 16 kHz: \( F(9,216)=5.59, P<0.001 \)). Animals co-treated with either 0.02 or 2 \( \mu \)g/ml \( \alpha \)-MSH showed significantly lower CAP thresholds, between day 0 and day 9, than those receiving saline co-treatment at 2 kHz (0.02 \( \mu \)g/ml: \( F(1,12)=5.77, P=0.033 \); 2 \( \mu \)g/ml: \( F(1,12)=6.82, P=0.023 \)) and 4 kHz (0.02 \( \mu \)g/ml: \( F(1,12)=4.22, P=0.062 \); 2 \( \mu \)g/ml: \( F(1,12)=7.21, P=0.020 \)), but not at 8 and 16 kHz, although a similar trend is present at these latter frequencies.

CM thresholds

Figure 4 shows the CM thresholds at 16 kHz, based on a 3 \( \mu \)V criterion. As the cochlear electrode was positioned at the round window, the CM generated in the basal turn contributed most to the signal, and hence only the CM elicited at 16 kHz stimulation was analyzed. CM threshold baseline values between day -6 and day -2 before cessation of cisplatin administration were averaged for each individual animal and set to zero. The CM thresholds at 16 kHz did not show significant recovery \( (F(9,216)=1.34, P=0.215) \). Still, animals treated with 0.02 \( \mu \)g/ml \( \alpha \)-MSH showed a significantly lower CM threshold shift, between day 0 and day 9, than those co-treated with saline \( (F(1,12)=7.88, P=0.016) \). The higher concentrations of \( \alpha \)-MSH did not show any significant effect at all.
Figure 4: Build-up and recovery of the CM threshold shift at 16 kHz (± S.E.M.) for the 4 different groups. The horizontal black bar represents the last 6 days of cisplatin administration. Day 0 represents the day that cisplatin administration was discontinued.

**CAP and CM amplitudes in relation to recovery and treatment**

In addition to threshold measurements it is informative to study the effect at higher stimulus levels. Figure 5 shows the CAP amplitudes obtained at stimulus levels of 78 to 87 dB SPL during and after cisplatin administration. Local perfusion with α–MSH resulted in less CAP amplitude reduction, especially at the lower frequencies. ANOVA from day 0 to day 9, performed on the logarithmically transformed CAP amplitudes, demonstrated main effects of frequency ($F_{(3,72)} = 19.49, P<0.001$) and time ($F_{(9,216)} = 20.39, P<0.001$) and a strong interaction between both factors ($F_{(27, 648)} = 3.30, P<0.001$). A separate analysis per frequency revealed a pronounced effect of co-treatment with 2 µg/ml α–MSH at 2 kHz ($F_{(1, 12)} = 9.34, P=0.010$). At the other frequencies there was only a main effect of time (recovery). Analysis of CM amplitudes at 16 kHz, 78 dB SPL (data not shown), starting at day 0, did not show an effect of co-treatment ($F_{(3,24)} = 1.03, P=0.40$), nor of time ($F_{(9,216)} = 1.66, P=0.10$).
Figure 5: Collapse and recovery at 2, 4, 8, and 16 kHz (± S.E.M.) of the CAP amplitude at 78-86 dB SPL stimulus level for the four different groups. The horizontal black bar represents the last 6 days of cisplatin administration. Day 0 represents the day that cisplatin administration was terminated.

**OHC loss in relation to co-treatment**

OHC counts for the right and left (implanted) ears of the four groups of animals are presented in Fig. 6. OHC losses were observed in the basal and middle turns of both left and right cochleas. OHC losses in the basal turns were more severe than in the middle ones. Remarkably, OHC loss in the left ears (with saline co-treatment) was more severe (10-15% remaining OHCs) than those in the right ears without cannula (50% remaining OHCs) \( \text{F}(1,23) = 5.20, \ P=0.032 \). This finding is in line with observations in chickens of Roberson et al. (2000). They found that cannula implantation itself may cause OHC death and can also cause potentiation of hair cell death induced by systemic gentamicin administration.

Since the cannula implantation itself seems to have a negative effect on hair cell survival, the effect of α–MSH co-treatment could only be compared within the left (implanted) ears. Statistical analysis showed a significant effect of
treatment in the basal turn of the ears co-treated with 2 µg/ml α−MSH as compared to the ears co-treated with saline (F (1,11)=6.05, P=0.032). Co-treatment with the lowest (0.02 µg/ml) and highest (20 µg/ml) concentration of α−MSH did not reach statistical significance (F (1,12)=3.94, P=0.071; F (1,10)=2.65, P=0.135, respectively).

Figure 6: Outer hair cell counts (percentage remaining OHCs) for the left (implanted) and right ear, as a function of position in the cochlear turns. Co-treatments: Open bar: saline; light-gray bar: 0.02 µg/ml α−MSH; dark-gray bar: 2 µg/ml α−MSH; black bar: 20 µg/ml α−MSH. Error bars represent S.E.M.

Discussion

α−MSH has a dose-dependent ameliorating effect on cisplatin intoxication

The first issue in this study was whether ameliorating effects of α−MSH on cisplatin ototoxicity, which were seen in previous studies (Heijmen et al., 1999; Wolters et al., 2003; Hamers et al., 2003), remain when α−MSH is applied exclusively perilymphatically as opposed to systemically. This study shows that intracochlear administration of 2 µg/ml α−MSH, with a mini-osmotic pump, indeed has an ameliorating effect on cisplatin-induced threshold shifts and the recovery in time (Figs. 2, 3) and also protects against OHC loss (Fig. 6). The group that received 0.02 µg/ml α−MSH showed small effects on both CAP and CM threshold shifts (Figs. 3, 4). With 20 µg/ml α−MSH in the pump, no statistically significant effects were found. Thus, the intermediate concentration (2 µg/ml in the pump) was the most optimal concentration in this study. Surprisingly and in contrast to the mirror experiment in which cisplatin was applied locally and α−MSH systemically (Wolters et al., 2003), we did not find an increase in the number of injections (days) necessary to evoke ototoxic
effects at any α-MSH concentration. This discrepancy can probably be explained by the large inter-animal variability associated with systemic cisplatin application (Klis et al., 2002) in combination with the relatively small size of the effect.

However, how can we explain that the highest concentration α-MSH applied in this study (20 µg/ml in the pump) is not effective while lower concentrations are? De Koning et al. (1986) found that the ACTH(4-9) analog ORG 2766 has an ameliorating effect on peripheral nerve regeneration with an inverted U-shaped dose-effect curve, i.e., low and high doses of ORG 2766 were inactive and only intermediate doses resulted in enhanced peripheral nerve recovery. We hypothesize that α-MSH, also an ACTH fragment, has a similar dose-effect curve for its ameliorating effects on cisplatin ototoxicity and that the highest dose results in a perilymphatic concentration that is well above the effective range. A point of concern with this hypothesis is that we do not know the validity of the comparison between both melanocortins, since we do not know the (sub)cellular mechanism by which both compounds exert their effects, neither with respect to enhancing peripheral nerve regeneration by ORG 2766, nor with respect to ameliorating cisplatin ototoxicity by α-MSH. Nevertheless, the present study has brought new information about the target of α-MSH.

α-MSH exerts its ameliorating effects on cisplatin-induced ototoxicity through a cochlear target

The most salient result in this study is that the positive effects we found with α-MSH, applied directly and exclusively to the cochlea, can almost only be explained by postulating a cochlear target for the compound. Especially in combination with the mirror study (Wolters et al., 2003), the possibility seems extremely unlikely that α-MSH works through interaction with a systemic factor or target. Given the strong possibility of a cochlear target, the question is of course which target? This is intimately related with the question about the target for cisplatin in the cochlea. On the basis of earlier data (Hamers et al., 1994; De Groot et al., 1997; Klis et al., 2000, 2002; O‘Leary and Klis, 2002), we hypothesize that cisplatin toxicity in the cochlea first occurs in the stria vascularis. Interference with strial function leads to a drop in EP (Klis et al., 2000, 2002; O‘Leary and Klis, 2002) and subsequently to a large increase of the CAP threshold. In a causally and yet unknown manner, these events might be related to OHC loss, progressing apically from the basal turn. Further, evidence is accumulating that suggests involvement of spiral ganglion cells (Zheng and Gao, 1996; Cardinaal et al., 2000b; Alam et al., 2000; Hamers et al., 2003), once again in an unknown relation to the strial and OHC effects described above. All these targets for cisplatin need to be considered as cochlear components with
which α-MSH might interfere.

α–MSH may exert its otoprotective effect by affecting the potency of the stria vascularis to counteract the effects of cisplatin. In principle, α–MSH might exert its protective action through activation of the melanocortin-1 receptor, which is commonly found in epidermal melanocytes. Intermediate cells, which are necessary for normal strial function and are involved in the generation of EP, have been identified as melanocytes (Motohashi et al., 1994). Also, it has been suggested that cochlear melanocytes are under α–MSH control (Meyer zum Gottesberge, 2000).

Since melanocortins have shown to exert neuroprotective effects in the peripheral sensory nerve system after cisplatin administration (De Koning et al., 1987; Hamers et al., 1993; Ter Laak et al., 2003), the otoprotective effect of the melanocortin α–MSH might also be related to interference with ganglion cells or the Schwann cells (Hol et al., 1994). Such an interference would explain the significantly smaller decrease in CAP amplitude at high sound levels in the 2 µg/ml α–MSH group (Fig. 5). Since the CAP at high stimulus levels is shown to depend on the viability of the ganglion cells, not so much on the viability of the OHCs (Hall, 1990; Schmiedt et al., 2002).

A direct effect of α–MSH on OHCs is, for lack of data about interference of melanocortins with hair cells, at present a purely hypothetical possibility.

Conclusion

The present study provides further evidence for the previously reported protective effect of α–MSH in cisplatin ototoxicity. The ototoxic effects of cisplatin were significantly reduced when the animals were co-treated with perilymphatically applied α–MSH. This preventive effect was dose-dependent and rather small, perhaps too small for clinical relevance. Since α–MSH was delivered directly to the cochlea, it is plausible that the ameliorating effect of α–MSH involves a cochlear target, possibly the strial melanocytes or the spiral ganglion cells.

Acknowledgements

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