Chapter 7

General discussion and summary
Melanocortins, peptides derived from AdrenoCorticoTropic Hormone (ACTH), such as α-MSH, and derivatives thereof, such as ORG 2766 and melanotan-II (MT-II), have been shown to protect against cisplatin-induced peripheral neuropathy. Previous experiments performed by our group have also shown protective effects of some of these peptides against cisplatin-induced ototoxicity. Based on these results, the aim of this thesis was to further characterize the protection efficacy of co-treatment with these neurotrophic peptides in relation to cisplatin-induced ototoxicity and, subsequently, to analyze the mechanisms involved. The studies performed were based on the following questions:

1) Do ORG 2766, α-MSH and MT-II delay the action of cisplatin?
2) Do the melanocortins enhance recovery after cisplatin treatment, and how?
3) Is there a neural component in cisplatin ototoxicity or is the effect confined to hair cells and stria vascularis?
4) Is the protective effect a direct local effect of the peptides or does the effect depend upon a systemic (intermediate) effect?
5) Can these melanocortin(-like) peptides be used in the clinic as otoprotective agents?

1) Do ORG 2766, α-MSH and MT-II delay the action of cisplatin?

Previous studies, in which a fixed number of injections of 1.5 or 2 mg/kg cisplatin was administered daily in combination with 75 µg/kg ORG 2766 (Hamers et al., 1994; Stengs et al., 1998b) or 75 µg/kg α-MSH (Heijmen et al., 1999), demonstrated a considerable number of co-treated animals that displayed preserved hearing after cessation of cisplatin and melanocortin treatment. This was not found in the cisplatin/saline controls. In the longitudinal animal model used in chapters 2, 3, and 4 of this thesis all animals were treated with cisplatin until a pronounced (criterion of ≥ 40 dB loss at 8 kHz) hearing loss occurred. Following the dichotomous results in the co-treated groups with the fixed dose experiments, we hypothesized that in the experiments with the longitudinal model the animals co-treated with ORG 2766 or α-MSH would require more injections of cisplatin than the saline co-treated animals to evoke the criterion threshold loss. Unexpectedly, neither α-MSH nor ORG 2766 consistently increased the mean number of cisplatin-injections (1.5 mg/kg/day) necessary to evoke this threshold loss. Also, the melanocortin MT-II (chapter 2) did not delay cisplatin ototoxicity. This can be explained by the large variability seen with cisplatin treatment in combination with a relatively
small effect. In the experiments described in this thesis, the most susceptible animals required 5 injections of cisplatin before reaching criterion threshold loss while the most resistant animal required 18 injections (without co-treatment). In such a noisy background only large delays can be proven to be statistically significant. Nevertheless, in several co-treated animals the onset of the ototoxic reactions seemed considerably delayed; the animals that required the highest cumulative dose of cisplatin were usually the co-treated ones, which might be an explanation of the dichotomy observed by Hamers et al. (1994), Stengs et al. (1998b), and Heijmen et al. (1999). These studies showed animals that were completely protected from cisplatin ototoxicity with normal auditory thresholds and no OHC loss but also animals that demonstrated increased thresholds similar to the animals treated with cisplatin alone. In an attempt to decrease the variability in susceptibility to cisplatin an alternative route of application was used in which we applied cisplatin directly into the cochlea (chapter 5). With this approach systemic factors that might be responsible for the large variability associated with systemic treatment could be eliminated. This experiment indeed showed a statistically significant delay in the group of animals that were systemically co-treated with α-MSH (75 µg/kg/day s.c.). However, when α-MSH was delivered directly into the cochlea via an osmotic pump system and cisplatin systemically (chapter 6) again only a trend was visible, which showed that some animals receiving the high dose of α-MSH (2 or 20 µg/ml in the pump) required more injections of cisplatin (2 mg/kg/day) to reach the criterion threshold shift than the animals with pure saline in the pump. Once again, we attribute this lack of effect of α-MSH to the higher variability associated with systemic application of cisplatin. However, we can not exclude causes associated with the different mode of application of α-MSH (local versus systemic).

2) Do the melanocortins enhance recovery after cisplatin treatment, and how?

Previous experiments by Klis et al. (2000, 2002) showed that when cisplatin treatment is stopped after reaching the criterion threshold shift, a pronounced recovery of hearing sensitivity occurs. This recovery reaches asymptotic levels after 10 days and is better at lower than at higher frequencies. Co-treatment with the melanocortin(-like) peptides ORG 2766 and α-MSH (chapter 3, 6) significantly changed the recovery after cisplatin treatment. Recovery of CAP threshold and CAP amplitude at high sound pressure levels was faster and
more complete. Furthermore, hair cell loss was significantly lower in the peptide co-treated groups. In contrast to the ameliorating effect of ORG 2766 and α-MSH, co-treatment with MT-II did not show a significant effect on recovery (chapter 2), although this compound is a more potent melanocortin-1 (MC1) and MC4-receptor agonist than α-MSH. Thus, since not all melanocortin(-like) peptides enhance recovery after cisplatin-induced ototoxicity it is difficult to identify the cochlear target(s) and the exact cellular mechanism involved in the otoprotective effects of the melanocortin(-like) peptides. Cisplatin causes pronounced damage to and even loss of OHCs (Komune et al., 1981; De Groot et al., 1997; Cardinaal et al., 2000a, b) and spiral ganglion cells (Zheng and Gao, 1996; Cardinaal et al., 2000b), and damage to the stria vascularis (Nakai et al., 1982; Kohn et al., 1988, 1997; Meech et al., 1998; Cardinaal et al., 2000a). Thus, one or more of these cochlear components might be involved in the recovery-process. Further characterization of the melanocortin-enhanced recovery process might bring us closer to the actual target of the melanocortins (and cisplatin itself) and with that the mechanism involved in the otoprotective action of melanocortins.

Co-treatment with α-MSH, or its derivatives, might enhance recovery from cisplatin-induced ototoxicity through activation of the MC1-receptor known to be present in melanocytes, for instance in the stria vascularis (Hilding and Ginzberg, 1977). The melanocytes in the stria, the so-called intermediate cells, have been suggested to be under α-MSH control (Meyer zum Gottesberge, 2000). However, co-treatment with the more potent MC1-receptor agonist MT-II did not show an enhancement of the recovery-process (chapter 2). Furthermore, ORG 2766 has no melanotrophic or corticotrophic activity (Greven and De Wied, 1973) and is known not to activate any of the five currently known MC-receptor subtypes (Adan et al., 1994, 1996). Since both α-MSH and ORG 2766 have similar protective and/or recovery enhancing effects in models of mechanical peripheral nerve damage (Bijlsma et al., 1984; Van der Zee et al., 1991) and ototoxicity (Hamers et al., 1994; De Groot et al., 1997; Stengs et al., 1998b; Heijmen et al., 1999) the question arises whether or not there is an as yet unidentified receptor for ORG 2766 that can also be activated by α-MSH.
3) Is there a relevant neural component in cisplatin ototoxicity or is the effect confined to hair cells and stria vascularis?

On the basis of earlier data (Hamers et al., 1994; De Groot et al., 1997; Klis et al., 2000), we hypothesized that cisplatin toxicity in the inner ear first occurs in the stria vascularis, since the endocochlear potential (EP) was decreased early in the process. Possibly, interference of cisplatin with strial function impairs its electrogenic activity, leading to a precipitous drop of the EP and a strong increase in CAP threshold (Hamers et al., 1994). In a causally and yet unknown manner, these events might be related to OHC loss, progressing apically from the basal turn. Discontinuation of cisplatin treatment enables the stria vascularis and thus the EP to recover, with concomitant CAP threshold recovery, provided that a sufficient number of OHCs have survived and are functional. The preliminary data in chapter 4 of this thesis, however, showed that the relation between EP recovery and CAP recovery may not be as strong as suggested above. The EP did not show recovery after 3 days, although significant recovery of the CAP threshold at 2 kHz was observed. However, CAP amplitudes at higher frequencies and at high stimulus levels did not show significant recovery in the limited time frame of chapter 4. In other words, we may have missed an essential part of the recovery process by limiting our time window to 3 days after cessation of the cisplatin treatment. We cannot rule out that the melanocortin(-like) peptides might exert their effect through another target than the stria. Both ORG 2766 (in vivo; Gerritsen van der Hoop et al., 1988; Muller et al., 1990; Hamers et al., 1993a) and α−MSH (in vitro; Windebank et al., 1994) have shown to protect from cisplatin-induced peripheral neuropathy. Thus, we cannot exclude the possibility that these compounds (partially) induce their protective effect in cisplatin-induced ototoxicity through modulation of a neural component. To date, some evidence for the involvement of the auditory nerve in cisplatin-induced hearing loss has been published. Both animal studies (Zheng and Gao, 1996; Alam et al., 2000; Cardinaal et al., 2000b) and a human study of post-mortem temporal bone material (Hinojosa et al., 1995) described morphological damage to the spiral ganglion cells in addition to OHC and strial damage.
4) Is the protective effect a direct local effect of the melanocortin-like peptides or does the effect depend upon a systemic (intermediate) effect?

So far we suggested that the melanocortin(-like) peptides ameliorate cisplatin-induced ototoxicity through a local (cochlear) target. However, since both cisplatin and the peptides were administered systemically in several studies (chapters 3 and 4) their effect could also have been mediated through a systemic effect. Such a systemic effect has been found earlier with the anti-oxidants, a group of sulphur containing compounds that are also known to prevent cisplatin-induced ototoxicity. The sulphur-groups in the anti-oxidants are known to bind irreversibly to heavy metals such as platinum. When both cisplatin and anti-oxidants are administered systemically, inactive sulphur-platinum-complexes are formed which are quickly excreted. This may result in a lowered systemic exposure to cisplatin, reducing its side effects but probably also its anti-tumor effect. Indeed, such a reduction in the systemic cisplatin concentration has been found when cisplatin was administered together with D-methionine (Ekborn et al., 2002). Since both ORG 2766 and α-MSH have been administered in our studies in doses at least 20 times smaller than the cisplatin dose, the protective effect of these peptides cannot be due to direct chemical interaction between cisplatin and α-MSH or ORG 2766.

Another possibility might be that melanocortin(-like) peptides exert their protective effect through enhancement of cisplatin clearance. The results in both chapter 5 and chapter 6 invalidate this hypothesis. In chapter 5 it was found that, despite the fact that cisplatin was administered locally through an osmotic pump system, systemic co-treatment with α-MSH significantly altered the number of days necessary to reach the criterion threshold shift. Furthermore, in chapter 6, in which α-MSH was administered directly into the ear and cisplatin systemically, we found small but significant effects on CAP threshold and OHC loss. Therefore, systemic effects of α-MSH on cisplatin excretion, or other absorption or transport factors can be excluded.
5) Can α-MSH and ORG 2766 be used in the clinic as an otoprotective agent?

With the results from this thesis we hoped to contribute to better understanding of cisplatin ototoxicity, which in turn might provide a key to ameliorate cisplatin ototoxicity in humans. With this knowledge we also hoped, eventually, to be able to generalize our results and find medical treatment for other acute cochlear insults, like those due to carboplatin treatment, aminoglycoside antibiotic treatment or even noise-induced hearing loss. The first part of this objective was reached. We showed that α-MSH does not delay the onset of cisplatin effects when cisplatin is applied systemically, but it enhances recovery and partly prevents OHC loss. The experiments with local application of cisplatin or α-MSH showed that α-MSH does not cause its effect through direct interaction with cisplatin or through stimulation of cisplatin excretion, but that the ameliorating effect of α-MSH probably involves a cochlear target, possibly the strial melanocytes or the spiral ganglion (nerve) cells. Thus, the results from this thesis confirm that α-MSH may be used to reduce cisplatin-induced ototoxicity. Also, the fact that α-MSH and its analogs show little side effects in clinical studies (Gerritsen van der Hoop, 1990; Wessels et al., 2000) pleads for introduction of the melanocortin-like peptides as a treatment for cisplatin-induced ototoxicity. However, this thesis shows that the protective effects of these peptides are rather small and that the effect varies considerably between individuals. This seriously hampers the introduction of these peptides as an otoprotective agent in patients. More research, especially about the dose-effect relationship of the peptides, has to be performed. Furthermore, the mechanism through which cisplatin exerts its ototoxic effects is such a complicated process that further research, especially at the morphological and molecular level, is necessary to reliably identify both the cochlear targets and the exact cellular mechanism involved in the otoprotective effect of the melanocortin(-like) peptides. The research performed within this thesis brought us a step closer to the unraveling of the mechanism of cisplatin-induced ototoxicity and ways and means to protect the ear against this highly potent ototoxicant.