British Journal of Pharmacology (1997) 120, 1561–1567

Different cardiovascular profiles of three melanocortins in conscious rats; evidence for antagonism between γ_2-MSH and ACTH-(1–24)

*Patricia Van Bergen *Jeroen A. Kleijne, *,†Dick J. De Wildt & [1,]*Dirk H.G. Versteeg

*Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Utrecht University, Universiteitsweg 100, 3584 CG Utrecht, and †National Institute for Public Health and Environmental Protection, Antonie van Leeuwenhoeklaan 9, 3720 BA Bilthoven, The Netherlands

1 We investigated the effects of [Nle^4,D-Phe^7]α-melanocyte-stimulating hormone (NDP-MSH), adrenocorticotropin-(1–24) (ACTH-(1–24)) and γ_2-MSH, three melanocortins with different agonist selectivity for the five cloned melanocortin receptors, on blood pressure and heart rate in conscious, freely moving rats following intravenous administration.

2 As was previously found by other investigators as well as by us, γ_2-MSH, a peptide suggested to be an agonist with selectivity for the melanocortin MC_3 receptor, caused a dose-dependent, short lasting pressor response in combination with a tachycardia. Despite the fact that NDP-MSH is a potent agonist of various melanocortin receptor subtypes, among which the melanocortin MC_3 receptor, it did not affect blood pressure or heart rate, when administered i.v. in doses of up to 1000 nmol kg^{-1}.

3 ACTH-(1–24) caused a dose-dependent decrease in blood pressure in combination with a dose-dependent increase in heart rate in a dose-range from 15 to 500 nmol kg^{-1}. The cardiovascular effects of ACTH-(1–24) were independent of the presence of the adrenals.

4 Pretreatment with ACTH-(1–24) caused a pronounced, dose-dependent parallel shift to the right of the dose-response curve for the pressor and tachycardiac effects of γ_2-MSH. The antagonistic effect of ACTH-(1–24) was already apparent following a dose of this peptide as low as 10 nmol kg^{-1}, which when given alone had no intrinsic hypotensive activity.

5 These results form further support for the notion that it is not via activation of one of the as yet cloned melanocortin receptors that γ-MSH-like peptides increase blood pressure and heart rate. The cardiovascular effects of ACTH-(1–24) seem not to be mediated by the adrenal melanocortin MC_2 receptors, for which ACTH-(1–24) is a selective agonist, or by adrenal catecholamines.

6 There appears to be a functional antagonism between ACTH-(1–24) and γ_2-MSH, two melanocortins derived from a common precursor, with respect to their effect on blood pressure and heart rate. Whether this antagonism plays a (patho)physiological role remains to be shown.

Keywords: γ_2-MSH (γ_2-melanocyte-stimulating hormone); ACTH-(1–24) (adrenocorticotropin-(1–24)); [Nle^4,D-Phe^7]α-MSH; blood pressure; heart rate; melanocortin receptor subtypes; functional antagonism

Introduction

It is now well documented that the pro-opiomelanocortin-derived melanotropin, γ_2-melanocyte-stimulating hormone (γ_2-MSH; amino acid sequence: Tyr-Val-Met-Gly-His-Phe-Arg-Trp-Asp-Arg-Phe-Gly), following its intravenous (i.v.) administration to conscious rats causes increases in blood pressure, heart rate and cerebral blood flow (Callahan *et al*., 1984; Klein *et al*., 1985; Sun *et al*., 1992; De Wildt *et al*., 1993; 1995; Versteeg *et al*., 1993; Van Bergen *et al*., 1995). Results of *in vitro* receptor studies have shown that γ_2-MSH binds and potently activates the melanocortin MC_3 Receptor (Gantz *et al*., 1993; Roselli-Rehfuss *et al*., 1993), whereas it has a low affinity for the other four known melanocortin receptor subtypes. The melanocortin MC_3 receptor is localized in various brain regions which are involved in cardiovascular regulations (Low *et al*., 1994). Recently we carried out a structure-activity analysis for the effect on blood pressure, heart rate and cerebral blood flow, testing a series of γ_2-MSH/ACTH-(4–10) analogues and fragments (Van Bergen *et al*., 1995; 1996), and postulated, based on discrepancies with *in vitro* data (Mountjoy *et al*., 1992; 1994; Chhajlani & Wikberg, 1992; Chhajlani *et al*., 1993; Roselli-Rehfuss *et al*., 1993; Gantz *et al*., 1993; Griffon *et al*., 1994; Low *et al*., 1994) that it is not likely that the γ-MSHs act via the melanocortin MC_3 receptor.

Since as yet, the possible role of other melanocortin receptor subtypes in cardiovascular regulatory mechanisms has not been studied, it was deemed of interest to investigate whether activation of the α-MSH (MC_1) and the ACTH-(1–24) (MC_2) receptor subtype results in changes in cardiovascular parameters. [Nle^4,D-Phe^7]α-MSH (NDP-MSH), an α-MSH analogue which is less susceptible to proteolytic degradation than its parent compound, has been shown to be the most potent agonist for the melanocortin MC_1 receptor subtype. However, in addition to the α-MSH receptor, which is the melanocortin MC_1 receptor (Chhajlani & Wikberg, 1992; Mountjoy *et al*., 1992), NDP-MSH activates the MC_3 receptor (Gantz *et al*., 1993), the MC_4 receptor (Mountjoy *et al*., 1994) and the MC_5 receptor (Chhajlani *et al*., 1993) as well. The corticotropin ACTH-(1–24) is a melanocortin receptor agonist which selectivity for the melanocortin MC_2 receptor (Mountjoy *et al*., 1992). Previously, it has been shown that this peptide causes a dose-dependent decrease in blood pressure in pithed and anaesthetized rabbits (Szabo *et al*., 1987; 1989; Ludbrook & Ventura, 1995). To our knowledge, there is only one preliminary study (Nakamura *et al*., 1976) in which a depressor effect of ACTH-(1–24) was demonstrated in rats. We, therefore, decided to investigate in more detail the cardiovascular effects of this melanocortin MC_2 receptor agonist in conscious rats as well.

[1]Author for correspondence at: Department of Medical Pharmacology, Rudolf Magnus Institute for Neurosciences, Universiteitsweg 100, 3584 CG Utrecht, The Netherlands.

On consideration of the preliminary results of Nakamura *et al*. (1976), which indicated a hypotensive effect of ACTH-(1–24), on the one hand and the well-established pressor effect of γ_2-MSH (Callahan *et al*., 1984; Klein *et al*., 1985; De Wildt *et al*., 1993; 1995; Versteeg *et al*., 1993; Van Bergen *et al*., 1995), on the other hand, a functional antagonism between these two peptides derived from the common prohormone might be anticipated. Such an antagonism has also been postulated to exist for melanocortins and endogenous opioids with respect to other systems (De Wied & Jolles, 1982; Eberle, 1988). Therefore, we examined, in order to investigate a possible (functional) antagonism, the interaction between γ_2-MSH and ACTH-(1–24) with respect to their effects on blood pressure and heart rate.

Methods

Animals

The experiments were carried out with male Wistar rats (U:WU cpb), weighing between 200–300 g. Before the operations the rats were housed three to a cage. Food pellets and tap water were provided to the rats *ad libitum*. Room temperature was kept at $20 \pm 1^{\circ}C$ with lights on from 6 h 00 min to 20 h 00 min and a relative humidity of 50–60%. After the operations the animals were housed individually under the same conditions.

The design of the study was approved by the Experimental Animal Boards of the Utrecht University and of the National Institute of Public Health and Environmental Protection.

Operation procedures

Cannulations were carried out 3 to 4 days before the experiment. Rats were anaesthetized with Nembutal in a dose of 0.11 ml 100 g^{-1} body weight, intraperitoneally. All details and modifications of the methods used have been described previously (Van Bergen *et al*., 1995). Briefly, for the measurement of blood pressure and heart rate cannulation of the aorta was performed. The cannula was inserted into the abdominal aorta, approximately 1 cm above the bifurcation to the lower extremities. Histoacryl blue was used to close the wound and to fix the cannula in place. The tubing was guided underneath the skin towards the skull. The cannula was filled with a PVP (50% polyvinylpyrrolidol (M = 25000); 500 iu ml^{-1} heparin) solution.

For i.v. administration of peptides the jugular vein was cannulated. The cannula was guided underneath the skin towards the skull and filled with saline.

Both cannulae were connected to a stainless steel connector and stoppered and fixed on the skull with dental cement. Post-operation treatment consisted of 0.05 ml Combi-kel 20/20, s.c. The animals were kept warm by either a lamp or a heating pad until they gained consciousness.

Measurement of blood pressure and heart rate

Arterial blood pressure and heart rate were measured by connecting the aortic line to a pressure transducer (Viggo-Spectramed, disposable DTX/plus). A PE100 tubing of a length sufficient to enable the rat to move around in its cage relatively undisturbed was connected to the stainless-steel loop of the aortic cannula on the head of the rat. The pressure transducer was connected to a pressure signal pre-amplifier/biotachometer (Instrument service, Utrecht University) coupled to a Wekagraph WK-812 AR recording system. Blood pressure and heart rate were recorded continuously at a chart speed of 10 mm min^{-1}.

Administration of peptides

For i.v. administration the peptides were dissolved in saline (0.9% NaCl). After a 45 min stabilisation period the peptides were infused into the jugular vein in the doses indicated in the Results section in a volume of 0.1 ml by means of a Braun infusion pump (Braun Perfusor) set at a rate of 0.5 ml min^{-1} during 12 s. Each infusion of a peptide was followed by infusion of 0.1 ml saline in order to flush the cannula. Therefore, a complete infusion procedure lasted 24 s. Saline served as vehicle control. Experiments were performed between 10 h 30 min and 15 h 00 min.

Experimental protocols

In the first experiment NDP-MSH and ACTH-(1–24) were administered i.v. at a time-interval of 10–15 min and mean arterial pressure (MAP) and heart rate (HR) were measured.

In the second experiment ACTH-(1–24) was administered i.v. to sham, adrenalmedullectomized (ADMX) and adrenalectomized (ADX) rats. For adrenalectomy, four days after cannulation of the abdominal aorta and the jugular vein, the rats were anaesthetized with ether and both adrenals were surgically removed. The ADX-group received 0.9% saline as drinking fluid. The sham-treated group received the same treatment except that the adrenals were left intact. Both groups were allowed to recover for two days. For adrenalmedullectomy, seven days before the cannulation of the abdominal aorta and jugular vein, the rats were anaesthetized with a mixture of halothane/O_2/NO_2. The medulla of each adrenal gland was squeezed through a nick made on its capsula. The rats did not require saline as drinking water during the recovery period. The completeness of the adrenodemedullation was checked ten days later by withdrawing blood from each rat for the measurement of plasma adrenaline. An aluminum oxide adsorption/extraction procedure was used followed by quantitation with high performance liquid chromatography (h.p.l.c.) with electrochemical detection. Only those rats which had plasma adrenaline levels under the detection limit of the method, which was 2 pg adrenaline per 100 μl plasma, were used. It takes at least 10 days for the adrenal cortex to regenerate and to produce basal levels of plasma corticosterone (Buckingham & Hodges, 1975; Holzwarth *et al*., 1980). On day 11 increasing doses of ACTH-(1–24) were administered i.v. with a time interval of 15 min.

In the third series of experiments the antagonism of ACTH-(1–24) on the pressor and tachycardiac effects of γ_2-MSH were examined. γ_2-MSH was administered i.v. and a dose-response analysis with respect to MAP and HR was performed before and after a bolus administration of ACTH-(1–24). The bolus doses of ACTH-(1–24) used were 1, 10, 100, 150 and 500 nmol kg^{-1} body wt. The time-interval between each administration was 5 min, which was sufficient for a complete recovery of blood pressure and heart rate after each dose of ACTH-(1–24) (see Figure 2).

In the fourth series of experiments the antagonism of ACTH-(1–24) on the cardiovascular effects of phenylephrine was investigated. A dose-response analysis was performed after i.v. administration of phenylephrine before and after a bolus administration of ACTH-(1–24) (150 nmol kg^{-1} body wt.). Saline was used as control. The time-interval between each administration was 5 min.

Calculation and statistical analysis

Mean arterial pressure was calculated according to the formula: $(2 \times P_d + P_s)/3$, in which P_d is diastolic pressure and P_s systolic pressure. For dose-response relationships the change in mean arterial pressure and heart rate was calculated at the time of maximal effect at compared to pre-injection values. In order to calculate ED_{50}- and E_{max}-values the data of the dose-response curves were fitted, by a non-linear steepest gradient method, to the Hill-equation: $E_D/E_{max} = [D^{n_H}([ED_{50}]^{n_H} + [D]^{n_H}]$, where E_D is the effect at dose D, E_{max} the (estimated) maximal effect at plateau, D dose, and ED_{50} effective dose required to produce half-maximal effect and n_H

a measure of cooperativity. The ED_{50} and E_{max} reflect a measure of potency and activity (α), respectively.

Basal values of MAP and HR were analysed by one-way analysis-of-variance (ANOVA) followed by Bonferroni as *post-hoc* test. Data of the dose-response curves were analysed by repeated-measures ANOVA followed by Student's *t* test as *post-hoc* test. The level of significance was set at the 95% confidence limit. All data are expressed as the mean ± s.e.mean.

Materials

Nembutal (sodium pentobarbitone, 60 mg ml^{-1}, benzylalcohol 9 mg ml^{-1}) was purchased from Sanofi B.V., Maassluis, The Netherlands; heparin (Tromboliquin, heparin sodium, 5000 iu ml^{-1}) from Organon Technika B.V., Boxtel, The Netherlands; Histoacryl blue (enbucrilate 1 g ml^{-1}) from B. Braun Melsungen A.G., Melsungen, Germany; Combi-kel 20/20 (procaine benzylpenicillin 200,000 iu ml^{-1}, dihydrostreptomycin sulphate 200 mg ml^{-1}) from Kombivet, Etten-Leur, The Netherlands; PVP (polyvinylpyrrolidol) from Merck, Darmstadt, Germany; and phenylephrine HCl from Sigma Chemical Co., St. Louis, U.S.A. NDP-MSH was purchased from Sigma Chemical Co., St. Louis, U.S.A., and γ_2-MSH from Bachem, Bubendorf, Switzerland. ACTH-(1–24) was kindly donated by Organon International B.V., Oss, The Netherlands.

Results

Experiment 1: effects of NDP-MSH, ACTH-(1–24) and γ_2-MSH administered i.v. to conscious rats

Basal MAP and HR values of the various groups were not significantly different from each other. Basal MAP varied from 95 ± 3 mmHg in the experiment with NDP-MSH ($n=6$) to 103 ± 3 mmHg in the experiment with ACTH-(1–24) ($n=6$). Basal HR varied from 337 ± 12 beats min^{-1} in the experiment with NDP-MSH to 353 ± 10 beats min^{-1} in the experiment with γ_2-MSH ($n=8$). Pre-administration values for MAP and HR were not significantly different before injection of each dose of peptide. Saline had no significant effect on MAP or HR.

I.v. administration of NDP-MSH (15–1000 nmol kg^{-1} body wt.) had no significant effect on MAP. ACTH-(1–24) (15–500 nmol kg^{-1} body wt.) induced a significant and dose-dependent decrease in MAP, whereas γ_2-MSH (5–150 nmol kg^{-1} body wt.) dose-dependently increased MAP (Figure 1a). The depressor response of ACTH-(1–24) was rather acute and maximal after 42 s following the start of administration. After 150 s MAP had returned to pre-administration values (Figure 2). A dose of 1000 nmol kg^{-1}, i.v. of ACTH-(1–24) caused a severe drop in blood pressure which the majority of the animals tested did not survive (results not shown).

NDP-MSH had no significant effect on HR. ACTH-(1–24) and γ_2-MSH caused a significant and dose-dependent increase in HR (Figure 1b). The tachycardia induced by ACTH-(1–24) was maximal after 90 s and HR returned to pre-administration values 300 s following the start of administration of the peptide (Figure 2).

Experiment 2: effects of ACTH-(1–24) administered intravenously to sham-operated, ADX, ADMX and control rats

The basal MAP of sham, ADX, ADMX and control (Experiment 1) rats did not differ significantly from each other. Basal MAP varied from 95 ± 2 mmHg for the ADMX group ($n=7$) to 109 ± 9 mmHg for the control group ($n=6$). Basal HR of the ADX group was slightly higher than of the other groups. Basal HR varied therefore from 351 ± 27 beats min^{-1} for the control group to 425 ± 12 beats min^{-1} for the ADX-

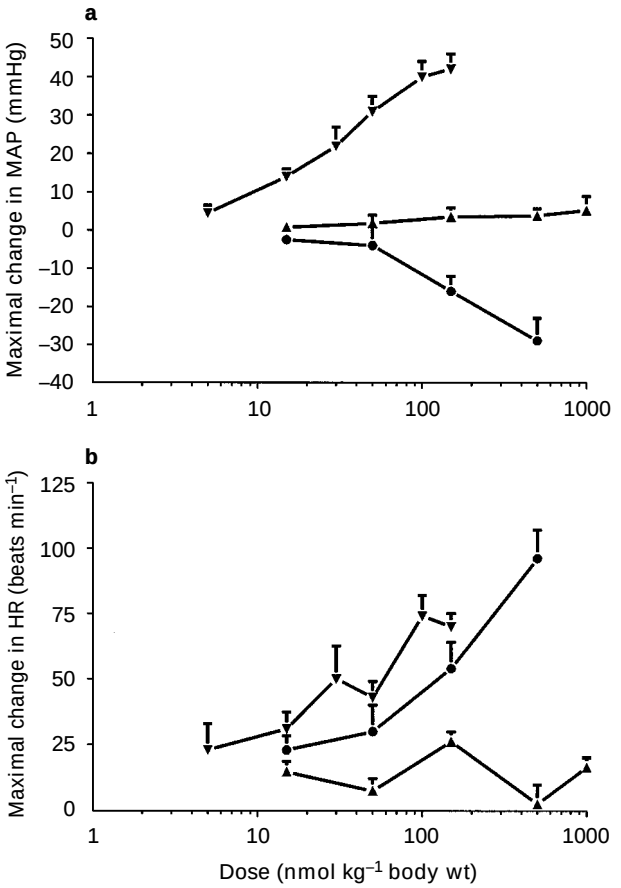

Figure 1 Dose-response curves for NDP-MSH (▲, $n=6$), ACTH-(1–24) (●, $n=6$) and γ_2-MSH (▼, $n=8$) following i.v. administration to conscious rats. Data are expressed as a maximal change in MAP (a) and HR (b), and represent the mean with vertical lines showing s.e.mean.

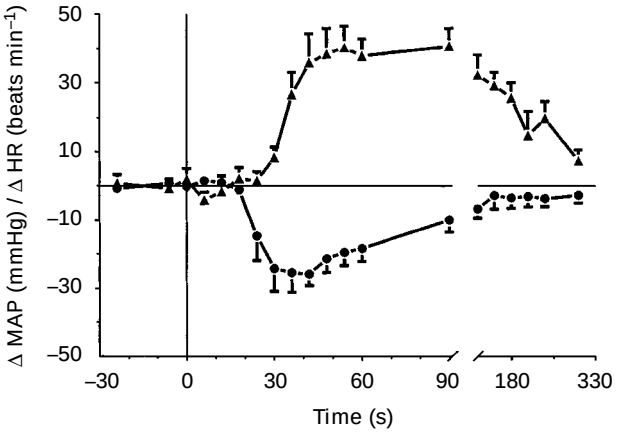

Figure 2 Time-effect relationship for ACTH-(1–24) (500 nmol kg^{-1} body wt.) for the maximal change of MAP (●) and HR (▲). On $t=0$ s ACTH-(1–24) was administered i.v. to conscious, freely moving rats ($n=6$). Vertical lines show s.e.mean.

group. Pre-administration values for MAP and HR were not significantly different before injection of each dose of peptide. Administration of the vehicle saline did not have a significant effect on MAP or HR.

Administration of ACTH-(1–24) (15–500 nmol kg^{-1} body wt.) induced a dose-dependent depressor response in all four groups (Figure 3a). No significant difference was observed between the four groups (treatment × dose interaction $F(12,100)=0.63$; $P>0.05$).

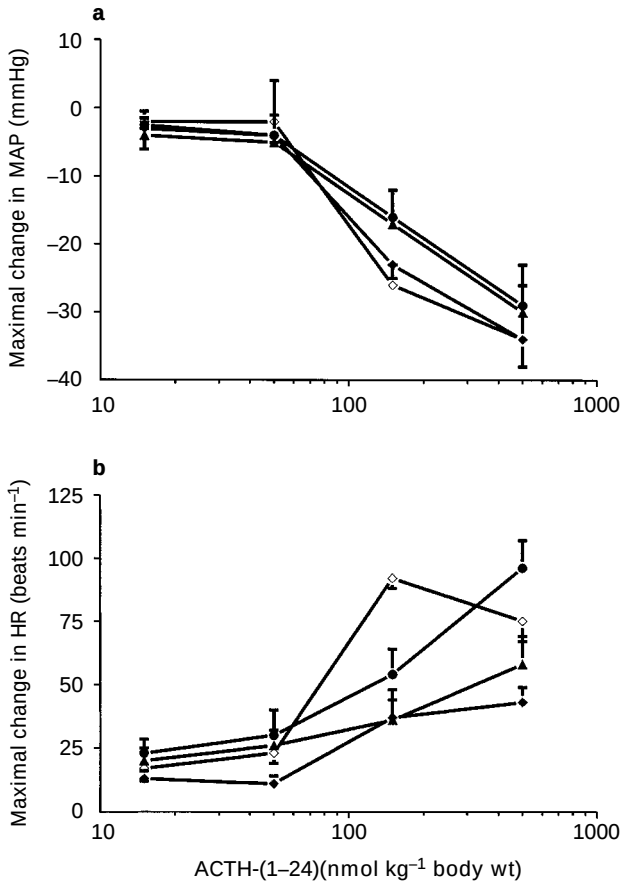

Figure 3 Dose-response relationship for ACTH-1–24) following i.v. administration to adrenalectomized (◆, $n=9$), adrenalmedullectomized (◇, $n=7$), sham (▲, $n=7$), and control (●, $n=6$) rats. Data are expressed as maximal change in MAP (a) and HR (b), and represent the mean with vertical lines showing s.e.mean.

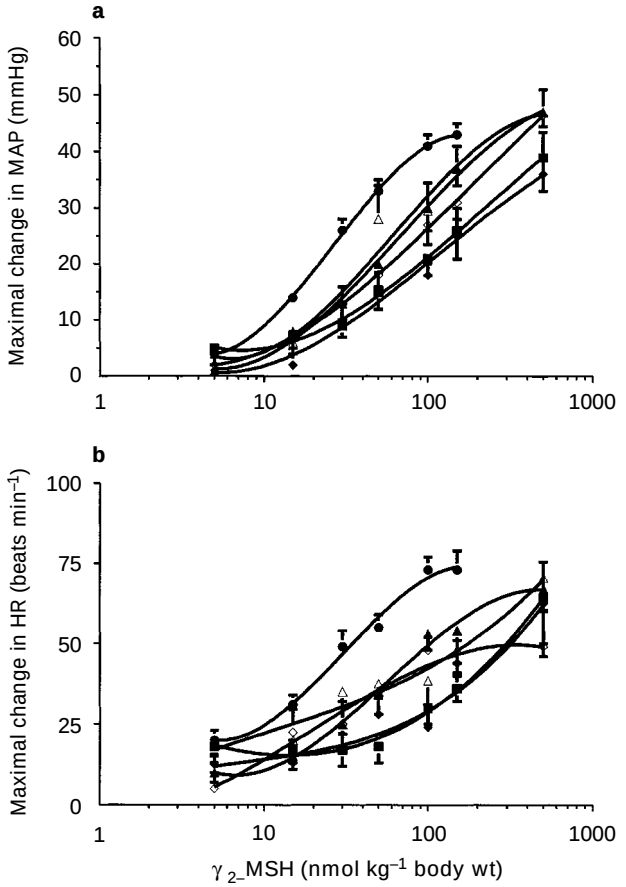

Figure 4 Effect of ACTH-(1–24) on the dose-response relationships for γ_2-MSH administered i.v. with respect to its effect on blood pressure and heart rate in conscious rats. The different doses were administered at 5 min intervals. Control γ_2-MSH, i.e. the dose-response curve for the effect of γ_2-MSH (5–150 nmol kg^{-1}) before ACTH-(1–24) administration (●, $n=34$), and, the dose-response curves for the effect of γ_2-MSH (5–500 nmol kg^{-1}) after pretreatment with ACTH-(1–24) in doses of 1 (△, $n=8$), 10 (▲, $n=7$), 100 (◇, $n=6$), 150 (◆, $n=6$) and 500 (■, $n=6$) nmol kg^{-1} body wt., respectively. Data are expressed as maximal change in MAP (a) and HR (b), and represent the mean with vertical lines showing s.e.mean.

Heart rate increased significantly after administration of ACTH-(1–24) (Figure 3b). A significant difference was observed between the four groups (treatment × dose interaction $F(12,100)=6.94$; $P<0.05$). A one-way analysis of variance showed that a dose of 150 nmol kg^{-1} the tachycardia in the ADMX group was significantly more pronounced than in the other groups. At a dose of 500 nmol kg^{-1} the increase in heart rate in both the ADX group and the sham group was less than that in the control group.

Experiment 3: antagonizing effects of ACTH-(1–24) on the pressor and tachycardiac responses of i.v. administered γ_2-MSH

The basal values of MAP and HR did not differ significantly between the various groups. Basal MAP varied between 93 ± 3 mmHg for the group treated with 10 nmol kg^{-1} ACTH-(1–24) ($n=7$) and 112 ± 5 mmHg, for the group treated with 150 nmol kg^{-1} ($n=6$). Basal HR varied between 353 ± 10 beats min^{-1} for the group treated with 1 nmol kg^{-1} ACTH-(1–24) ($n=8$) and 382 ± 14 beats min^{-1} for the group treated with 500 nmol kg^{-1} ($n=6$).

I.v. administration of γ_2-MSH (5–150 nmol kg^{-1} body wt.) induced a strong and dose-dependent increase in MAP (Figure 4a). There was no significant difference between the different groups before ACTH-(1–24) administration (treatment × dose interaction $F(20,145)=1.05$, $P>0.05$). The ED_{50} value for the effect of γ_2-MSH was 28 ± 3 nmol kg^{-1} and E_{max} was 44 ± 3 mmHg. ACTH-(1–24) in doses of 10, 100, 150 and 500 nmol kg^{-1} body wt. caused a parallel shift of the dose-pressor response of γ_2-MSH to the right, without significantly modifying the maximal pressor response of γ_2-MSH (Figure 4a). A dose of ACTH-(1–24) of 1 nmol kg^{-1} had no significant effect on the dose-pressor curve of γ_2-MSH. Saline instead of ACTH-(1–24) had no significant effect (data not shown).

A dose-dependent tachycardia was seen after i.v. administration of γ_2-MSH (Figure 4b). Again, there was no significant difference between the different groups before ACTH-(1–24) administration (treatment × dose interaction $F(20,140)=1.44$; $P>0.05$). As was the case for blood pressure, ACTH-(1–24) in doses of 100, 150 and 500 nmol kg^{-1} body wt. significantly shifted the tachycardiac response of γ_2-MSH to the right (Figure 4b). Saline had no significant effect on the dose-response curves of γ_2-MSH (data not shown).

The antagonizing effect of ACTH-(1–24) could only be demonstrated with a time-interval of 5 min between each dose of peptide. With an interval of 15 min no significant shifts in the dose-response curve of γ_2-MSH were seen with 500 nmol kg^{-1} ACTH-(1–24) (data not shown).

Experiment 4: antagonizing effects of ACTH-(1–24) on the pressor response of i.v. administered phenylephrine

Since we were interested whether the competitive antagonism of ACTH-(1–24) on the cardiovascular responses of γ_2-MSH was specific, we investigated the effect of ACTH-(1–24) on the

pressor response of phenylephrine, an α_1-adrenoceptor agonist. ACTH-(1–24) (150 nmol kg^{-1}) significantly shifted the pressor response of phenylephrine to the right in a parallel manner (Figure 5a). No significant effect was observed on the bradycardia (Figure 5b). Pretreatment with saline instead of ACTH-(1–24) had no effect on the pressor and bradycardiac response of phenylephrine (Figure 5a and b).

Discussion

In order to elucidate the involvement and, therefore, the possible role of various subtypes of the melanocortin receptor in cardiovascular regulations, we studied the effects of three melanocortin receptor agonists, γ_2-MSH, NDP-MSH and ACTH-(1–24), on blood pressure and heart rate.

Results of *in vitro* studies have shown that γ_2-MSH preferentially binds and activates MC_3 receptors (Gantz *et al*., 1993). As demonstrated previously (Callahan *et al*., 1984; Klein *et al*., 1985; Sun *et al*., 1992; De Wildt *et al*., 1993; 1995; Versteeg *et al*., 1993; Van Bergen *et al*., 1995). i.v. administration of γ_2-MSH causes a dose-dependent increase in blood pressure and heart rate. This suggests that the melanocortin MC_3 receptor is involved in the pressor and chronotropic actions of γ_2-MSH (Low *et al*., 1994). However, our findings with a rather selective melanocortin receptor agonist, NDP-MSH, which binds and stimulates the melanocortin receptor of the MC_1, MC_3, MC_4 and MC_5 subtypes (see below), makes this possibility less likely. Systemic administration of NDP-MSH in doses of up to 1000 nmol kg^{-1} did not result in significant changes in either blood pressure or heart rate. This observation seems to be in agreement with previous findings with α-MSH. In conscious rats this melanotropin did not affect blood pressure and heart rate in doses of up to 150 nmol kg^{-1} (De Wildt *et al*., 1993) or 5 mg kg^{-1} (Klein *et al*., 1985). NDP-MSH is a potent and, due to its reduced susceptibility to proteolytic breakdown, relatively long acting analogue of α-MSH (Sawyer *et al*., 1980). Results of *in vitro* receptor studies have shown that not only the melanocortin MC_1 receptor, which is the α-MSH receptor (Chhajlani & Wikberg, 1992; Mountjoy *et al*., 1992), but also the melanocortin MC_3 (Gantz *et al*., 1993), MC_4 (Mountjoy *et al*., 1994) and MC_5 (Chhajlani *et al*., 1993) receptors are more potently activated by NDP-MSH than by α-MSH. Of these receptors the melanocortin MC_3 and MC_4 are localized in various brain regions involved in cardiovascular regulation (Low *et al*., 1994; Mountjoy *et al*., 1994). Among these regions are the circumventricular organs, to which circulating peptides have access (Weindl, 1973), such as the periventricular region of the third ventricle of the anterior hypothalamus (the AV3V region). These periventricular regions appear to be critically involved in integrating neural and humoral aspects of cardiovascular regulation (see Brody *et al*., 1984) and might be the site via which systemically administered melanotropins cause sympathetic drive to increase (Gruber & Callahan, 1989). The finding that NDP-MSH, which is an agonist with an affinity for the melanocortin MC_3 receptor higher than that of γ_2-MSH, does not alter blood pressure and heart rate might be explained as indicating that it is via a melanocortin receptor subtype other than the MC_3 receptor that the γ-MSHs act to increase blood pressure and heart rate. Recently, we reached this same conclusion based on discrepancies in the results of structure-activity studies with a series of γ-MSH/ACTH-like peptides for their effects on blood pressure and cerebral blood flow (Van Bergen *et al*., 1995; 1996) and on adenosine 3′:5′-cyclic monophosphate (cyclic AMP) production in cells expressing the melanocortin MC_3 receptor (Van Bergen *et al*., unpublished observations). Alternatively, it might be that NDP-MSH is without effect, because it simultaneously activates two subtypes of the melanocortin receptor which mediate opposite effects on cardiovascular paradigms. That NDP-MSH is biologically inert *in vivo* is not a likely explanation for its lacking effects on blood pressure and heart rate, since it has been shown to have effects on grooming behaviour, learning and memory, visual performance end nerve regeneration (for a review, see Eberle, 1988).

Further experiments with the corticotropin ACTH-(1–24), which has selectivity for the melanocortin MC_2 receptor (Mountjoy *et al*., 1992), were carried out in order to investigate whether this receptor subtype plays a role in the cardiovascular regulations. In contrast to γ_2-MSH, i.v. administration of ACTH-(1–24) caused a decrease in blood pressure with a concomitant increase in heart rate in the conscious rat. We used doses of up to 500 nmol kg^{-1} to demonstrate the dose-dependency of the effects of this corticotropin. In the cock 100 μg kg^{-1}, which corresponds to 34 nmol kg^{-1}, of this corticotropin causes a small decrease in MAP of 8 mmHg (Jaques, 1965). In the anaesthetized rabbit, infusion of ACTH-(1–24) in doses of up to 1 μg kg^{-1} min^{-1} for 3 to 10 min was shown to cause a decrease in blood pressure and a tachycardia (Szabo *et al*., 1989), whereas in the conscious rabbit doses of the peptide of up to 3.4 nmol kg^{-1} had a slight, but significant effect on MAP and HR (Ludbrook & Ventura, 1995). ACTH-(1–39) and [Gly^1]ACTH-(1–18)-NH_2, a derivative with full adrenocorticotrophic activity, also caused a decrease in blood pressure in the rabbit and cat (Ueda *et al*., 1970).

It has been demonstrated that the melanocortin MC_2 receptor is potently stimulated by ACTH-(1–24) (Mountjoy *et al*., 1992). This receptor is localised in the adrenal gland, predominantly in the zona fasciculata (glucocorticoid production) (Griffon *et al*., 1994). Also the melanocortin MC_5 receptor is localised predominantly in the zona glomerulosa (aldosterone production) of the adrenal gland. ACTH-(1–24) is slightly more potent than NDP-MSH in stimulating this latter receptor

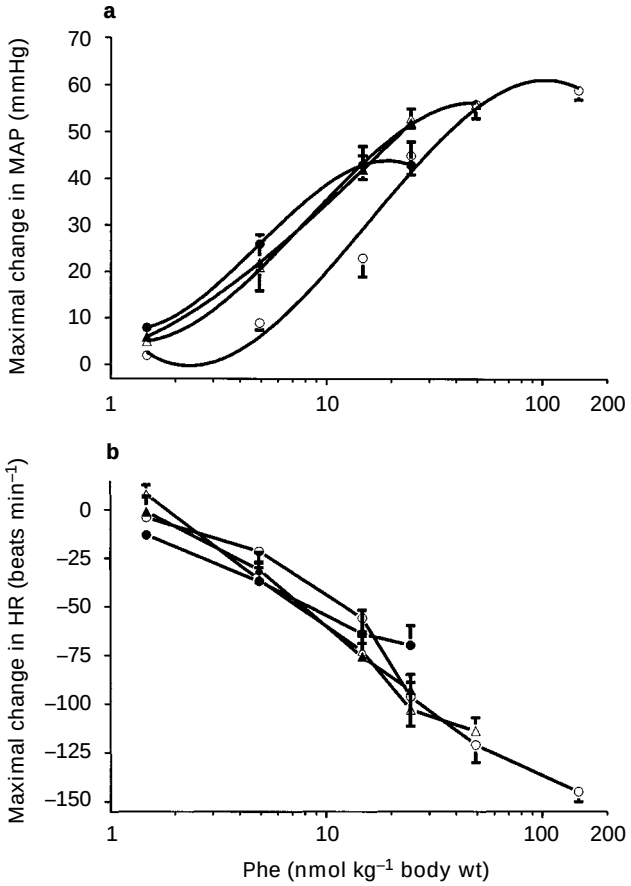

Figure 5 Effect of ACTH-(1–24) on the dose-response relationship for phenylephrine (Phe) administered i.v. with respect to its effect on blood pressure and heart rat in conscious rats. Controls (solid symbols) and the effect of phenylephrine after treatment with either saline (△, $n=5$) or ACTH-(1–24), 150 nmol kg^{-1} body weight (○, $n=6$). Data are expressed as maximal change in MAP (a) and HR (b), and represent the mean with vertical lines showing s.e.mean.

(Griffon *et al*., 1994). Since ACTH-(1–24) was fully effective in decreasing blood pressure in adrenalectomized rats, our results confirm the preliminary observations made by Nakamura *et al*. (1976). It, therefore, appears likely that neither the melanocortin MC_2 nor the MC_5 receptor in the adrenals mediate the cardiovascular effects of this peptide. We have no explanation for the unexpected difference in effect of ACTH-(1–24) on heart rate between control rats and sham rat.

In the rabbit the decrease in blood pressure after ACTH-(1–24) administration appears to be the result of direct systemic vasodilatation, since in the pithed rabbit with and without electrical stimulation the corticotropin is equally effective in inducing a depressor effect (Szabo *et al*., 1987; 1989). It is tempting to speculate that in the rat ACTH-(1–24) exerts its depressor effect by this same mechanism of action. As yet no data are available to support this assumption. In any case, the effect of ACTH-(1–24) is not mediated by the adrenal cortex or medulla, since then peptide is equally effective in adrenalectomized, adrenal-medullectomized and sham-operated rats. In addition, it is well known that adrenaline induces a pressor response, whereas adrenal steroids are devoid of cardiovascular activity (Szabo *et al*., 1987; 1989; Ludbrook & Ventura, 1995). These findings imply that the depressor effect of ACTH-(1–24) is most likely an extra-adrenal action as has been suggested by Nakamura *et al*. (1976).

The tachycardia induced by ACTH-(1–24) is presumably caused by activation of the baroreceptor response due to the decrease in MAP (Szabo *et al*., 1989; Ludbrook & Ventura, 1995). However, ACTH-(1–24) increases myocardial sensitivity to noradrenaline (Mehrabani & Bassett, 1988), suggesting that direct effects of the corticotropin on the heart might also contribute to the tachycardia. It is worthy of note that in the anaesthetized rabbit ACTH-(1–24) induces, in addition to a depressor response and tachycardia, an increase in renal sympathetic nerve activity and plasma noradrenaline concentration (Szabo *et al*., 1989). The increase in plasma noradrenaline is the result of the facilitatory action of ACTH-(1–24) on presynaptic noradrenaline release via activation of melanocortin supposedly localized at postganglionic sympathetic neurones (Göthert, 1984; Szabo *et al*., 1987).

γ_2-MSH caused strong dose-dependent increases in MAP and heart rate, as was previously found (Callahan *et al*., 1984; Klein *et al*., 1985; Sun *et al*., 1992; Versteeg *et al*., 1993; De Wildt *et al*., 1993; 1995; Van Bergen *et al*., 1995). The finding that the effects of ACTH-(1–24) on blood pressure are opposite to those of γ_2-MSH, prompted us to investigate whether there is antagonism between these homologous peptides. ACTH-(1–24) caused a parallel shift to the right of the dose-response curve for the effects of γ_2-MSH on blood pressure and heart rate. It should be noted that the dose-response curves were established when blood pressure and heart rate changes due to ACTH-(1–24), if any, had returned to basal values. The antagonistic effect of ACTH-(1–24) was already apparent following a dose of the peptide as low as 10 nmol kg^{-1}, which by itself has no intrinsic hypotensive activity. The antagonistic effect could only be demonstrated with a dose-time interval of 5 min and not 15 min, which supposedly is due to the short half-life of ACTH-(1–24). The antagonism is competitive in nature and relatively strong. A comparison of ED_{50} values for γ_2-MSH in absence and presence of the highest antagonistic dose of ACTH-(1–24), showed that a shift to the right over one decade could be measured. The competitive nature of the relatively strong antagonism at relatively low doses of ACTH-(1–24) suggests that it probably takes place at the (heterogeneous) melanocortin receptor level. However, antagonism not related to melanocortin receptors should also be considered, as ACTH-(1–24) inhibited the pressor effect of the adrenoceptor agonist phenylephrine as well. However, the shift of the phenylephrine dose-response curve in the presence of a high dose of ACTH-(1–24) was considerably less (< factor 2) as compared with the antagonism towards γ_2-MSH-induced cardiovascular effects. Although antagonism at the melanocortin receptor level is most likely, partly aspecific antagonism between ACTH-(1–24) and γ_2-MSH cannot be ruled out.

As low a dose as 10 nmol kg^{-1} ACTH-(1–24) significantly antagonized the pressor response of γ_2-MSH, which suggests that this antagonism might be operating under physiological and/or pathophysiological conditions. In view of the close relationship of both melanocortins, which act on the same group of receptors, with intrinsic opposite effects on blood pressure, one it tempted to postulate a functional antagonism between these two melanocortins. However, at present no data are available concerning cardiovascular effects of these compounds when released endogenously. The exact role that these peptides play in the cardiovascular regulation under (patho)-physiological conditions is subject to further investigations. Antagonism between peptides derived from the prohormone pro-opiomelanocortin is not a new phenomenon. It has been demonstrated for melanocortins, such as ACTH, and opioids. It has been postulated that melanocortins are the endogenous, physiological antagonists of opioids (for a review, see Eberle, 1988). Because melanocortins and opioids are localized in the same cells, are derived from the same prohormone, share the same target centres, and are processed and released in varying amounts and ratios depending on the (patho)physiological conditions both in CNS and periphery, it is assumed that melanocortins and opioids constitute a co-ordinated, well-balanced peptidergic system (De Wied & Jolles, 1982; Eberle, 1988). Furthermore, a functional antagonism seems to exist between interleukin-1 and α-MSH. Interleukin-1 is thought to be a major pyrogenic agent and α-MSH has been shown to antagonize some of the effects induced by interleukin-1 on the immune system (see Eberle, 1988).

In summary, the present results show that NDP-MSH, which is a potent agonist of various melanocortin receptor subtypes, is devoid of effects on blood pressure and heart rate after i.v. administration to conscious rats. These results form further support for the notion that it is not via activation of one of the as yet discovered subtypes of the melanocortin receptor that γ-MSH-like peptides increase blood pressure and heart rate. The results further show that ACTH-(1–24) causes a dose-dependent decrease in blood pressure, in combination with a tachycardia, effects which are independent of the presence of the adrenal cortex and medulla. These effects, therefore, seem not to be mediated by the adrenal melanocortin MC_2 receptor, for which ACTH-(1–24) is a selective agonist, or by adrenal catecholamines. ACTH-(1–24) and γ_2-MSH, two peptides derived from a common precursor and sharing a partial homology in amino acid sequence, thus have opposite effects on the blood pressure in conscious rats. In addition, there appears to be a functional antagonism between these two melanocortins.

We gratefully acknowledge the skilful technical assistance of Mr Henk De Lang and Mr Henk A. Spierenburg. We also thank Dr E.L. de Beer, Department of Medical Physiology, Utrecht University, for the data-fit-programme and Dr H.J.A. Wijnne, Center of Biostatistics, Utrecht University, for advice on statistical analysis of the data.

References

BRODY, M.J., FABER, J.E., MANGIAPANE, M.L. & PORTER, J.P. (1984). The central nervous system and the prevention of hypertension. In *Handbook of Hypertension*, Vol. 4: *Experimental and Genetic Models of Hypertension*, ed. De Jong, W., pp. 474–494, Amsterdam: Elsevier Science Publishers.

BUCKINGHAM, J.C. & HODGES, J.R. (1975). Interrelationships of pituitary and plasma corticosterone during adrenocortical regeneration in rat. *J. Endocrinol.*, **67,** 411–417.

CALLAHAN, M.F., KIRBY, R.F., LYMANGROVER, J.R., JOHNSON, A.K. & GRUBER, K.A. (1984). Cardiovascular mechanisms of gamma $(\gamma)_2$-MSH. *Clin. Exp. Hypertension*, **A6,** 1727–1730.

CHHAJLANI, V., MUCENIECE, R. & WIKBERG, J.E.S. (1993). Molecular cloning of a novel human melanocortin receptor. *Biochem. Biophys. Res. Commun.*, **195,** 866–873.

CHHAJLANI, V. & WIKBERG, J.E.S. (1992). Molecular cloning of the human melanocyte stimulating hormone receptor cDNA. *FEBS Lett.*, **309,** 417–420.

DE WIED, D. & JOLLES, J. (1982). Neuropeptides derived from pro-opiomelanocortin: behavioral, physiological, and neurochemical effects. *Physiol. Rev.*, **62,** 976–1059.

DE WILDT, D.J., KASBERGEN, C.M. & VERSTEEG, D.H.G. (1995). Effect of γ_2-melanocyte-stimulating hormone on cerebral blood flow in rats. *J. Cardiovasc. Pharmacol.*, **25,** 898–905.

DE WILDT, D.J., KRUGERS, H., KASBERGEN, C.M., DE LANG, H. & VERSTEEG, D.H.G. (1993). The hemodynamic effects of γ_2-melanocyte-stimulating hormone and related melanotropins depend on the arousal potential of the rat. *Eur. J. Pharmacol.*, **233,** 157–164.

EBERLE, A.N. (1988). *The Melanotropins. Chemistry, Physiology and Mechanisms of Action.* New York: S Karger Publishers.

GANTZ, I., KONDA, Y., TASHIRO, T., SHIMOTO, Y., MIWA, H., MUNZERT, G., WATSON, S.J., DELVALLE, J. & YAMADA, T. (1993). Molecular cloning of a novel melanocortin receptor. *J. Biol. Chem.*, **268,** 8246–8250.

GÖTHERT, M. (1984). Facilitatory effect of adrenocorticotropic hormone and related peptides on Ca2+-dependent noradrenaline release from sympathetic nerves. *Neuroscience*, **11,** 1001–1009.

GRIFFON, N., MIGNON, V., FACCHINETTI, P., DIAZ, J., SCHWARTZ, J.-P. & SOKOLOFF, P. (1994). Molecular cloning and characterization of the rat fifth melanocortin receptor. *Biochem. Biophys. Res. Commun.*, **200,** 1007–1014.

GRUBER, K.A. & CALLAHAN, M.F. (1989). ACTH-(4–10) through γ-MSH: evidence for a new class of central autonomic nervous system-regulating peptides. *Am. J. Physiol.*, **257,** R681–R694.

HOLZWARTH, M.A.., SHINSAKO, J. & DALLMAN, M.F. (1980). Adrenal regeneration. Time course, effect of hypothalamic hemi-island and response to unilateral adrenalectomy. *Neuroendocrinology*, **31,** 168–176.

JAQUES, R. (1965). Non-specific effects of synthetic corticotrophin polypeptides. *Int. Arch. Allergy*, **28,** 221–230.

KLEIN, M.C., HUTCHINS, P.M., LYMANGROVER, J.R. & GRUBER, K.A. (1985). Pressor and cardioaccelerator effects of gamma MSH and related peptides. *Life Sci.*, **36,** 769–775.

LOW, M.J., SIMERLY, R.B. & CONE, R.D. (1994). Receptors for the melanocortin peptides in the central nervous system. *Curr. Opin. Endocrinol. Diabetes*, 79–88.

LUDBROOK, J. & VENTURA, S. (1995). ACTH-(1–24) blocks the decompensatory phase of the haemodynamic response to acute hypovolaemia in conscious rats. *Eur. J. Pharmacol.*, **275,** 267–275.

MEHRABANI, P.A. & BASSETT, J.R. (1988). Effect of (1–24) adrenocorticotrophin on the uptake of ^{3}H-norepinephrine by the rat atria. *Pharmacol. Biochem. Behav.*, **30,** 391–396.

MOUNTJOY, K.G., MORTRUD, M.T., LOW, M.J., SIMERLY, R.B. & CONE, R.D. (1994). Localization of the melanocortin-4 receptor (MC4-R) in neuroendocrine and autonomic control circuits in the brain. *Mol. Endocrinol.*, **8,** 1298–1308.

MOUNTJOY, K.G., ROBBINS, L.S., MORTRUD, M.T. & CONE, R.D. (1992). The cloning of a family of genes that encode the melanocortin receptors. *Science*, **257,** 1248–1251.

NAKAMURA, M., MATSUDA, S., UEDA, M. & TANAKA, A. (1976). Depressor effect of synthetic peptides related to ACTH on blood pressure in rats. *Experientia*, **32,** 368–369.

ROSELLI-REHFUSS, L., MOUNTJOY, K.G., ROBBINS, L.S., MORTRUD, M.T., LOW, M.J., TATRO, J.B., ENTWISTLE, M.L., SIMERLY, R.B. & CONE, R.D. (1993). Identification of a receptor for γ melanotropin and other proopiomelanocortin peptides in the hypothalamus and limbic system. *Proc. Natl. Acad. Sci. U.S.A.*, **90,** 8856–8860.

SAWYER, T.K., SANFILIPPO, P.J., HRUBY, V.J., ENGEL, M.H., HEWARD, C.B., BURNETT, J.B. & HADLEY, M.E. (1980). 4-Norleucine, 7-D-phenylalanine-α-melanocyte-stimulating hormone. A highly potent α-melanotropin with ultralong biological activity. *Proc. Natl. Acad. Sci. U.S.A.*, **77,** 5754–5758.

SZABO, B., HEDLER, L., LICHTWALD, K. & STARKE, K. (1987). ACTH increases noradrenaline release in pithed rabbits with electrically stimulated sympathetic outflow. *Eur. J. Pharmacol.*, **136,** 391–399.

SZABO, B., HEDLER, L. & STARKE, K. (1989). Facilitation of the release of noradrenaline: an extra-adrenal effect of adrenocorticotropic hormone. *Resuscitation*, **18,** 229–242.

SUN, X.Y., FENG, Q.P., GONG, Q.L., EDVINSSON, L. & HEDNER, T. (1992). Cardiovascular and renal effects of γ_2-MSH in spontaneously hypertensive and normotensive rats. *Am. J. Physiol.*, **262,** R77–R84.

UEDA, M., MATSUDA, S., KAWAKAMI, M. & TAKEDA, H. (1970). Depressor mechanism of synthetic ACTH (50022). *Jap. J. Pharmacol.*, **20,** 585–598.

VAN BERGEN, P., JANSSEN, P.M.L., HOOGERHOUT, P., DE WILDT, D.J. & VERSTEEG, D.H.G. (1995). Cardiovascular effects of γ-MSH/ACTH-like peptides: structure-activity relationship. *Eur. J. Pharmacol.*, **294,** 795–803.

VAN BERGEN, P., VAN DER VAART, J.G.M., KASBERGEN, C.M., VERSTEEG, D.H.G. & DE WILDT, D.J. (1996). Structure-activity analysis for the effects of γ-MSH/ACTH-like peptides on cerebral hemodynamics in rats. *Eur. J. Pharmacol.*, **318,** 357 – 368.

VERSTEEG, D.H.G., KRUGERS, H., MEICHOW, C., DE LANG, H. & DE WILDT, D.J. (1993). Effect of ACTH-(4–10) and γ_2-MSH on blood pressure following intracerebrovascular and intracisternal administration. *J. Cardiovasc. Pharmacol.*, **21,** 907–911.

WEINDL, A. (1973). Neuroendocrine aspects of circumventricular organs. In *Frontiers in Neuroendocrinology*, ed. Ganong, W.F. & Scapagnini, U., pp. 3–32. Oxford: Oxford University Press.

(Received November 26, 1996
Accepted January 9, 1997)