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α-Methyldopa induces a naltrexone-insensitive antinociception and hypomotility in rats

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1 This study served to investigate whether endogenous opioid peptides play a role in the putative antinociceptive and the sedative actions of α-methyldopa.

2 In conscious normotensive rats, α-methyldopa induced hypotension, starting around 1 h and reaching a maximum 3–4 h after administration. Pretreatment with naltrexone resulted in an inhibition of α-methyldopa-induced hypotension.

3 α-Methyldopa dose-dependently increased hot plate latency which became evident after a 4 h lag period and reaching a maximum effect at 6 h. The antinociceptive effect of α-methyldopa was not affected by naltrexone.

4 In a small open field, α-methyldopa dose-dependently suppressed locomotion and sniffing behaviour. These effects of α-methyldopa were apparent 1 h after administration and were naltrexone-insensitive.

5 No changes in the level of β-endorphin-like immunoreactivity in plasma and cerebrospinal fluid were observed after administration of α-methyldopa.

6 The results indicate that endogenous opioid peptides are involved in the hypotensive action of α-methyldopa but not in α-methyldopa-induced hypomotility and antinociception.

Introduction

The antihypertensive substance α-methyldopa reduces blood pressure after neuronal uptake and metabolization to α-methylnoradrenaline, an α_2-adrenoceptor agonist, by a centrally mediated effect on the autonomic nervous system (for review see Henning, 1984). The α_2-receptors, that are stimulated by α-methylnoradrenaline, are presumably located in the nucleus tractus solitarii (NTS) (Nijkamp & De Jong, 1975; De Jong & Nijkamp, 1976).

Evidence is accumulating showing that endogenous opioid peptides play a role in the hypotensive mechanism of action of α-methyldopa and clonidine. Kunos and coworkers were the first to report that the opiate receptor antagonist, naloxone, inhibited clonidine-induced hypotension (Farsang & Kunos, 1979) and partially reversed the hypotension induced by α-methyldopa (Farsang *et al.*, 1980) in spontaneously hypertensive rats. In normotensive rats, intracisternal (Van Giersbergen & De Jong, 1988) and intra-NTS (Petty & De Jong, 1984) pretreatment with naloxone resulted in an inhibition of the decrease in blood pressure induced by administration via the same route of α-methyldopa and α-methylnoradrenaline, respectively. Since an antiserum against β-endorphin also inhibited α-methyldopa- (Van Giersbergen *et al.*, 1989b) and clonidine-induced hypotension (Ramirez-Gonzalez *et al.*, 1983; Van Giersbergen *et al.*, 1989a), this opioid peptide might be the endogenous ligand for the opiate receptors that are blocked by naloxone. Microinjection of low doses (pg range) of β-endorphin into the NTS resulted in hypotension (Petty & de Jong, 1982). It was postulated that the release of β-endorphin, probably in the area of the NTS, is a step in the process by which α_2-adrenoceptor agonists reduce blood pressure (Kunos *et al.*, 1981). In fact, both clonidine and α-methylnoradrenaline stimulate the release of β-endorphin-like immunoreactivity (β-ELIR) from brainstem slices of spontaneously hypertensive rats (Kunos *et al.*, 1981). This was an *in vitro* study and, as yet, no data are available on the effects of α-methyldopa on β-ELIR levels in normotensive rats *in vivo*.

It has been suggested that a release of endogenous opioid peptides in the central nervous system is also involved in the antinociceptive action of clonidine (Tchakarov *et al.*, 1985; Mastrianni *et al.*, 1989). Such an action of clonidine has been long known (Schmitt *et al.*, 1973; Paalzow & Paalzow, 1976) and recently it was demonstrated that naloxone inhibited clonidine-induced antinociception in both normotensive and hypertensive rats (Tchakarov *et al.*, 1985). Since α-methylnoradrenaline could also induce a release of β-ELIR (see above), α-methyldopa might cause a naltrexone-sensitive antinociception. At the moment, no data are available on the putative antinociceptive effects of α-methyldopa.

In the present study, using normotensive rats, we investigated, besides α-methyldopa-induced hypotension, the postulated antinociceptive effect and the sedative action of α-methyldopa. Our attention focussed on the question whether endogenous opioid peptides are involved in the observed effects. In addition, the effects of α-methyldopa on the level of β-ELIR in cerebrospinal fluid (CSF) and plasma were studied.

Methods

Animals

Male Wistar rats of an inbred strain (Cpb: WU) weighing between 200 and 250 g were used for the experiments. They were housed under standard conditions (room temperature 21°C, light on from 06 h 00 min till 20 h 00 min) and received food pellets and water *ad libitum*.

Blood pressure measurement

The left femoral artery was cannulated under ether anaesthesia (Van Giersbergen & De Jong, 1988). After surgery, the rats were housed individually. On the days of the experiments, 1 and 3 days after cannulation of the artery, the rats were brought to the experimental room and allowed 30 min to adjust to the environment. Then, the catheter was connected to a Statham pressure transducer (P23AC) and arterial pressure was displayed on a Grass polygraph (model 79C and 7D).

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Heart rate was calculated from the blood pressure recording using a 6 s period of fast chart speed. Basal blood pressure and heart rate were determined during a period of 30 min.

To assess the antagonistic activity of naltrexone on the hypotension induced by α-methyldopa, rats were pretreated with this opiate antagonist (2 mg kg^{-1}, i.p.) or with saline followed 20 min later by the i.p. injection of 200 mg kg^{-1} of α-methyldopa. The animals were used twice. On the second experimental day, 3 days after surgery, the pretreatment was reversed. Thus, the effect of α-methyldopa on blood pressure was tested twice in each animal, once after saline and once after naltrexone. Arterial blood pressure was recorded continuously for 8 h after the administration of α-methyldopa and each 30 min heart rate was determined.

Collection of CSF

For this purpose a stainless steel cannula was implanted into the cisterna magna according to a method developed by Bouman & Van Wimersma Greidanus (1979). Briefly, rats were anaesthetized with Hypnorm (fentanyl 0.2 mg ml^{-1}; fluanison 10 mg ml^{-1}; 1 ml kg^{-1}, i.m.) and the head was fixed in a stereotaxic apparatus. After exposure of the skull, three holes were drilled, two for stainless steel screws and one for the cannula. The cannula was fixed with dental acrylic cement (Biofast D.L., Dental CCO, Richmond, N.Y., U.S.A.). After surgery the rats were housed individually. Each day after cannulation the rats were given a sham i.p. injection, the needle was introduced but no fluid was injected, followed by the collection of approximately 50 μl of CSF using PE-100 tubing attached to a 100 μl syringe. This procedure was followed for two reasons: (1) To keep the cannula patent, and (2) to accustom the rats to this procedure. The experiments in which CSF was collected were carried out 4 and 7 days after the intracisternal cannulation.

Behavioural tests

For behavioural testing the rats were transferred to a sound-attenuated room at least 1 h before the start of the experiment. The actual behavioural testing was performed in all experiments between 14 h 00 min and 18 h 00 min. The animals were used once. To assess the activity of rats they were placed in a circular perspex test cage (diameter 19.5 cm; height 28.5 cm), the bottom of which was divided into 4 sections of equal size. In this small open field locomotion (number of sections explored), rearing, standing on the hind legs, grooming (number of demonstrations) and sniffing (total time) were determined during a 3 min observation period (Van Ree & Wolterink, 1981). The hot plate method (Eddy & Leimbach, 1953) was used to investigate the antinociceptive action of α-methyldopa. The rats were placed on a hot plate which was kept at 54°C and the latency of the first nociceptive response was measured. Licking of the paws or fast stamping was the criterion of the response. Cutoff time in the absence of a response was 30 s. The rats were placed on the hot plate 60 and 30 min before the first injection to determine basal latency, and once after the injection to assess the effects of the administered substance(s).

Protocols

Two groups of rats with an intracisternal cannula were prepared. On the day of the experiment the rats were transported to the quiet experimental room and allowed 1 h to adjust. Then, α-methyldopa (200 mg kg^{-1}, i.p.) or saline was injected. The first group was used to study the effect of α-methyldopa on the CSF level of β-ELIR. At several times after administration (15–240 min) CSF was collected. For each time point a separate group of rats was used and the animals were used twice. On the first day, 4 days after the intracisternal cannulation, the rats received α-methyldopa or saline. This treatment was reversed during a second experiment 3 days later. CSF was collected in ice-chilled tubes, centrifuged at 4°C for 15 min at 2000*g* and the supernatant was stored at −20°C until further processing. In the second group of animals the effect of α-methyldopa on plasma β-ELIR content was determined. At several time points after drug or saline administration (30–240 min) the rats were decapitated and trunk blood was collected in ice-chilled polypropylene tubes containing 0.2 ml 50 mM EDTA. Plasma was prepared by centrifugation at 2000*g* for 10 min at 4°C and stored at −20°C until further processing.

The effects of α-methyldopa (200 mg kg^{-1} i.p.) on small open field behaviour and hot plate latency were tested between 1 and 8 h after injection. A separate group of rats, receiving α-methyldopa or saline, was used for each time point. To construct a dose-response curve rats were treated with α-methyldopa (50–400 mg kg^{-1} i.p.) or saline and behavioural testing was performed 4 h after drug administration.

To test whether the observed behavioural and antinociceptive effects of α-methyldopa were mediated by activation of opiate receptors, the influence of naltrexone was tested. A dose of 2 mg kg^{-1} i.p. of naltrexone was chosen since this dose effectively antagonized both the antinociception induced by morphine as measured on the hot plate (data not shown) and α-methyldopa-induced hypotension (see Results). Naltrexone or saline was administered 30 min before the injection of α-methyldopa or 30 min before the behavioural testing which was performed 4 h after α-methyldopa administration. It has been shown that the dose of α-methyldopa in relation to the dose of antagonist is an important factor in establishing an antagonistic action of naltrexone (Van Giersbergen & De Jong, 1988). Therefore, doses of 100 and 200 mg kg^{-1} of α-methyldopa, that induced reproducible effects, were used in the small open field and hot plate experiments, respectively.

Extraction of β-endorphin-like immunoreactivity from plasma and radioimmunoassay

β-Endorphin and related peptides were extracted and concentrated from plasma according to the method described by Ratcliffe & Edwards (1971) with slight modifications (Barna *et al.*, 1988). The recovery of the extraction procedure was monitored by simultaneous extraction of ^{125}I-labelled β-endorphin and amounted to 82%. Radioimmunoassays were performed as described elsewhere (Barna *et al.*, 1988). The antiserum (B4) was raised in rabbits against human β-endorphin. This antiserum was directed against the (9–16) sequence of the β-endorphin molecule. Synthetic camel β-endorphin was used as standard and iodinated camel β-endorphin as tracer. With the B4 antiserum the following cross-reactivities (expressed as % on mass basis) were obtained: human β-LPH, 39%; camel β-endorphin, 100%; γ-endorphin, 391%; α-endorphin, 170%; α-MSH, 0.7%. Cross-reactivity with ACTH, [Met^5]enkephalin, dynorphin A(1–13), α-methyldopa and α-methylnoradrenaline was <0.2%. All samples were assayed in duplicate.

Drugs

(±)-Naltrexone HCl (Du Pont Pharmaceuticals, Wilmington, Delaware, U.S.A.) and (−)-α-methyldopa (Merck, Sharpe and Dohme, Haarlem, The Netherlands) were dissolved in physiological saline (0.9% NaCl) and injected i.p. in a volume of 1 and 12 ml kg^{-1} body weight, respectively.

Statistical analysis

Values for mean arterial pressure (MAP) and heart rate are shown in Figure 2 as a change from the base-line values. MAP was calculated from the blood pressure recording by the equation:

$$\text{MAP} = \text{diastolic blood pressure} + 2/5\,(\text{systolic} - \text{diastolic blood pressure}).$$

All values presented are means ± s.e. The data shown in Figures 1, 3, and 4, and in the tables are analyzed by one-way

analysis of variance (ANOVA) followed by Student-Newman-Keuls test; Student's t test was used for the other data. A value of P less than 0.05 was considered to indicate a significant difference.

Results

Blood pressure experiment

A dose of 200 mg kg^{-1} of α-methyldopa potently decreased blood pressure as can be seen in Figure 1. This hypotension did not become evident until 2 h after administration and reached a maximum between 3 and 4 h after injection. Blood pressure returned to base-line value at about 8 h after administration (data not shown). The fall in blood pressure was accompanied by a tachycardia which was maximal at 1 h after drug administration and, thereafter, heart rate began to return to the base-line value (Figure 1). Pretreatment with the opiate receptor antagonist naltrexone (2 mg kg^{-1}, i.p.) resulted in a significant inhibition of α-methyldopa-induced hypotension (-7.7 ± 4.5 mmHg as compared to -21.4 ± 1.8 mmHg in saline-treated controls, $P < 0.05$), whereas the tachycardia was not affected by naltrexone (Figure 1). Administration of naltrexone or saline alone did not result in changes in blood pressure or heart rate (data not shown).

Hot plate experiments

Administration of α-methyldopa (200 mg kg^{-1}, i.p.) to male Wistar rats resulted in an increased hot plate latency but the effect did not become evident until 4 h after administration (Figure 2). The maximal response was reached 6 h after drug administration and the effect was still present 8 h after injection (Figure 2). Figure 3 shows a dose-response curve of the antinociceptive action of α-methyldopa. Doses of 50 and 100 mg kg^{-1} tended to increase hot plate latency. This increase became significantly different from the saline-treated rats at a dose of 200 mg kg^{-1} (Figure 3). Treatment of the animals with naltrexone 30 min before the administration of α-methyldopa, or 30 min before the hot plate test, 3.5 h after α-methyldopa, had no influence on α-methyldopa-induced antinociception (Figure 4). The hot plate latency of animals treated with naltrexone and saline did not differ from that of saline-saline-treated controls (Figure 4).

Small open field experiments

Administration of α-methyldopa (200 mg kg^{-1}, i.p.) markedly depressed small open field behaviour. As is illustrated in Figure 5, this dose of α-methyldopa significantly decreased locomotion and sniffing while rearing and grooming were also depressed, but this depression did not reach, in general, statistical significance. These effects of α-methyldopa were already apparent 1 h after drug administration, reached a maximum 3–4 h after injection and lasted for at least 8 h (Figure 5). The behavioural effects of α-methyldopa were dose-dependent. As can be seen in Table 1, a dose of 50 mg kg^{-1} decreased total sniffing time and tended to inhibit locomotion at 4 h after injection. Both parameters were inhibited by the two higher doses of α-methyldopa (100 and 200 mg kg^{-1}). Rearing and grooming were not significantly affected. In contrast to α-methyldopa-induced hypotension, pretreatment with naltrexone (data not shown) as well as treatment with this opiate antagonist 3.5 h after administration of α-methyldopa (Table 2) did not influence the suppression of locomotion and sniffing caused by α-methyldopa. The small open field behaviour remained unchanged after the injection of naltrexone alone (Table 2).

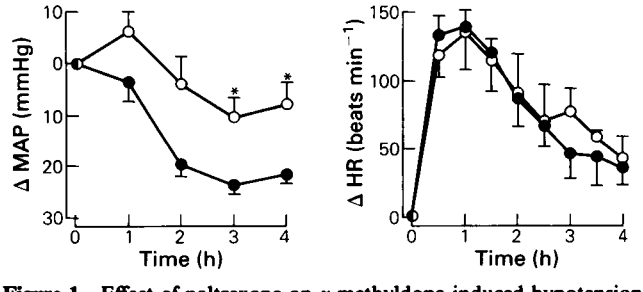

Figure 1 Effect of naltrexone on α-methyldopa-induced hypotension and tachycardia in conscious rats. Naltrexone (○, 2 mg kg^{-1}, i.p.) or saline (●) was injected 20 min before the administration of α-methyldopa 200 mg kg^{-1}, i.p. Mean arterial pressure (MAP, mmHg) was recorded continuously for 4 h and heart rate (HR, beats min^{-1}) was determined once every 30 min. Data represent change from base-line value and are presented as means with s.e. indicated by vertical bars. *$P < 0.05$ (ANOVA followed by Student-Newman-Keuls test) as compared to saline-pretreated controls.

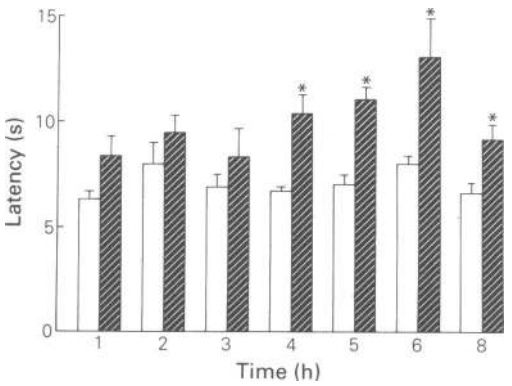

Figure 2 Time course of the antinociceptive action of α-methyldopa. Rats were placed on a hot plate at 54°C, 60 and 30 min before the injection of α-methyldopa (hatched columns, 200 mg kg^{-1}, i.p.) or saline (open columns) to determine the basal response latency and once after drug administration. The latency (s) after treatment is depicted and each column represents mean (with s.e. indicated by vertical bars) of a separate group of 6–7 animals. *$P < 0.05$ (Student's t test) as compared to saline-treated controls.

Effect of α-methyldopa on CSF and plasma β-endorphin-like immunoreactivity levels

At several time points after the administration of α-methyldopa (200 mg kg^{-1}, i.p.), corresponding to the time

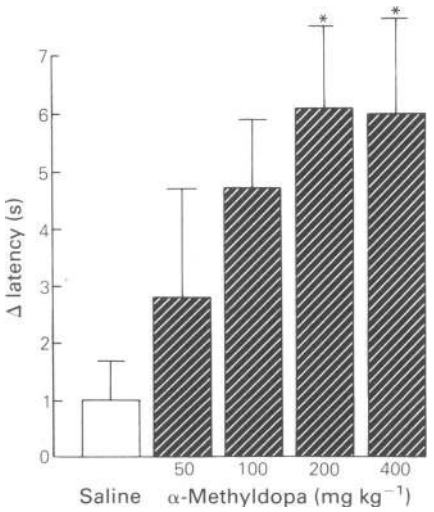

Figure 3 Dose-response curve of the analgesic action of α-methyldopa. Rats were placed on a hot plate at 54°C 60 and 30 min before the injection of α-methyldopa (50–400 mg kg^{-1}, i.p.) or saline to determine the basal response latency and 4 h after drug administration. The change in latency (s) as compared to the basal response was determined and each dose group consisted of 6–7 animals. Data represent change from the basal response latency (s) and are presented as means (s.e. indicated by vertical bars). *$P < 0.05$ (ANOVA followed by Student-Newman-Keuls test) as compared to saline-treated controls.

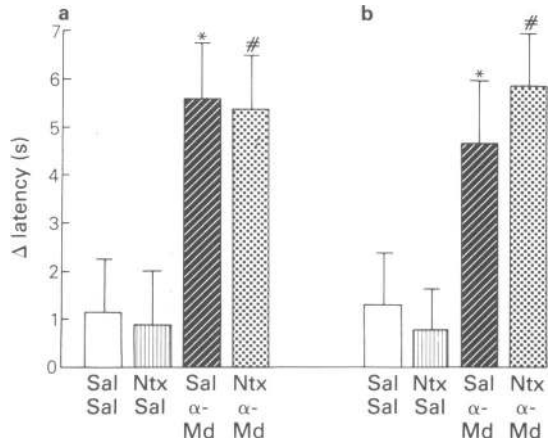

Figure 4 Effect of naltrexone on the antinociceptive action of α-methyldopa. Rats were treated with naltrexone (Ntx, 2 mg kg^{-1}, i.p.) or saline (Sal) 30 min before the injection of α-methyldopa (α-Md, 200 mg kg^{-1}, i.p.) or saline (a), or with naltrexone or saline 3.5 h after α-methyldopa (b), and tested on the hot plate 4 h after α-methyldopa administration. Basal hot plate latencies were determined 60 and 30 min before the first injection. The latency (s) to the first response was determined and each group consisted of 6–7 animals. Data represent the change from the basal response latency and are presented as means (s.e. shown by vertical bars). $^{*,\#}P < 0.05$ (ANOVA followed by Student-Newman-Keuls test) as compared to saline- or naltrexone-treated controls, respectively.

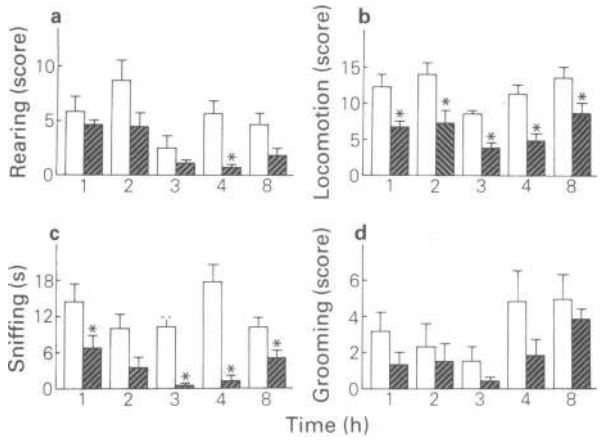

Figure 5 Time course of the behavioural effects of α-methyldopa. Naive rats were treated with α-methyldopa (hatched columns, 200 mg kg^{-1}, i.p.) or saline (open columns) and were placed in a small open field. During 3 min, (a) rearing, (b) locomotion, (d) grooming and the time spent on (c) sniffing (s) were scored. Data are presented as means, each column represents a separate group of 6–7 animals (vertical bars show s.e.). $^{*}P < 0.05$ (Student's t test) as compared to saline-treated controls.

curve of the hypotensive effect of α-methyldopa, the concentration of β-ELIR in both plasma and CSF was determined. Neither in the first hour, 15, 30, 45 and 60 min after administration, when the interaction between α-methyldopa and the endogenous opioids is suspected to take place (Van Giersbergen & De Jong, 1988), nor at the time point of the maximal fall in blood pressure, 3–4 h after drug administration, was the level of β-ELIR in CSF affected by α-methyldopa (data not shown). The concentration of β-ELIR in CSF ranges from 221–587 pg ml^{-1} in the saline-treated controls. Throughout the study, the concentration of β-ELIR in plasma was lower than in CSF (119 ± 3.6 pg ml^{-1} as compared to 352 ± 20.9 pg ml^{-1}). α-Methyldopa had no significant effect on the concentration of β-ELIR as measured in plasma 60, 90, 120 and 240 min after administration of α-methyldopa (data not shown). The level of β-ELIR in plasma tended to be increased by α-methyldopa 30 min after the injection of the antihypertensive drug (data not shown).

Discussion

The present data indicate that activation of opiate receptors does not play a role in the hypomotilic and antinociceptive action of α-methyldopa. On the other hand, naltrexone was able to inhibit α-methyldopa-induced hypotension which confirms earlier findings (Van Giersbergen & De Jong, 1988) of an involvement of endogenous opioids in the cardiovascular effects of this centrally acting antihypertensive drug.

Pretreatment with naltrexone resulted in an inhibition of the fall in blood pressure after i.p. administration of α-methyldopa, whereas the accompanying tachycardia was not affected by the opiate antagonist. Earlier studies have shown that intracisternal administration of opiate antagonists and of a β-endorphin antiserum inhibited the hypotension and bradycardia induced by α-methyldopa administered via the same route (Van Giersbergen & De Jong, 1988; Van Giersbergen *et al.*, 1989b). In contrast to central administration, peripheral injection of α-methyldopa causes tachycardia which gradually returns to the base-line value. This tachycardia is probably the result of a direct action of α-methyldopamine, a metabolite of α-methyldopa, on the heart (Van der Maas *et al.*, 1986) and endogenous opioids do not mediate this response, as shown by the lack of effect of naltrexone. Since the combination of the dose of naltrexone and the dose of α-methyldopa is a critical factor in demonstrating a naltrexone-α-methyldopa interaction (Van Giersbergen & De Jong, 1988), it is important to note that the doses of these substances used in the present study do reveal such an interaction with respect to blood pressure.

To our knowledge this is the first time that an antinociceptive action of α-methyldopa has been reported. The lag period before the appearance of the increase in hot plate latency might be the reason why an antinociceptive effect of α-methyldopa has not been reported earlier. The antinociceptive effect of α-methyldopa is dose-dependent over a dose-range similar to the range of doses that induce hypotension. However, the time course of the antinocicepetive response appears to be different from that of the cardiovascular events, suggesting different underlying mechanisms. This notion is further supported by the observation that naltrexone did not influence α-methyldopa-induced antinociception whereas it inhibited α-methyldopa-induced hypotension. These data, therefore, indicate that endogenous opioid peptides are not involved in the antinociceptive action of α-methyldopa.

Table 1 Dose-response relationship of the behavioural suppressive effect of α-methyldopa (50–200 mg kg^{-1}, i.p.)

Treatment	*Dose* (mg kg^{-1})	*Locomotion* (score)	*Sniffing* (s)	*Rearing* (score)	*Grooming* (score)
Saline		12.1 ± 1.6	11.5 ± 2.6	3.4 ± 1.3	1.6 ± 0.7
α-Methyldopa	50	9.5 ± 1.4	3.4 ± 1.3*	3.6 ± 1.3	4.3 ± 1.0
α-Methyldopa	100	7.8 ± 0.9*	1.4 ± 0.5*	1.9 ± 0.4	1.4 ± 0.4
α-Methyldopa	200	5.8 ± 1.1*	0.3 ± 0.2*	2.0 ± 0.7	1.3 ± 0.6

Rats were tested in a small open field 4 h after administration of α-methyldopa. Data are presented as means ± s.e. ($n = 8$). $^{*}P < 0.05$ as compared to saline-treated controls (ANOVA followed by Student-Newman-Keuls test).

Table 2 Effect of naltrexone (2 mg kg^{-1}, i.p.) administered 30 min before the small open field test on the behavioural response to α-methyldopa (100 mg kg^{-1})

Treatment	*Dose* (mg kg^{-1})	*Locomotion* (score)	*Sniffing* (s)	*Rearing* (score)	*Grooming* (score)
Saline + saline		12.8 ± 1.0	17.4 ± 1.4	5.0 ± 1.2	3.2 ± 0.9
Naltrexone + saline	2	11.7 ± 0.8	21.3 ± 3.0	5.3 ± 0.8	1.7 ± 1.7
Saline + α-Methyldopa	100	7.8 ± 0.8*	2.7 ± 1.2*	3.0 ± 1.0	3.8 ± 1.7
Naltrexone + α-Methyldopa	2 100	7.0 ± 0.9†	0.8 ± 0.3†	2.5 ± 0.7	1.8 ± 0.5

Rats were tested in a small open field 4 h after administration of α-methyldopa. Data are presented as means ± s.e. ($n = 6$).
*$P < 0.05$ as compared to saline-saline-treated controls.
†$P < 0.05$ as compared to naltrexone-saline-treated controls (ANOVA followed by Student-Newman-Keuls test).

Several studies have demonstrated an antinociceptive action of clonidine in mice (Schmitt *et al.*, 1973) and other animal species (Skingle *et al.*, 1982), and in man (Tamsen & Gordh, 1984; Coombs *et al.*, 1985). This effect of clonidine appears to be centrally mediated (Skingle *et al.*, 1982). In the literature the evidence for a clonidine-naloxone interaction regarding antinociception is controversial. Several reports show no effect of naloxone (Paalzow & Paalzow, 1976; Fielding *et al.*, 1978; Dennis *et al.*, 1980) whereas in others naloxone inhibited the antinociceptive effect of clonidine (Lin *et al.*, 1980; Tchakarov *et al.*, 1985; Nakamura *et al.*, 1988; Mastrianni *et al.*, 1989). In part, these controversial results may be explained by the use of high doses of clonidine, that are in excess of the dose range that induces hypotension, which might mask a possible involvement of endogenous opioids. Tchakarov *et al.* (1985) demonstrated in rats that naloxone inhibited the antinociceptive response to low doses but not to a high dose of clonidine. On the other hand, a great variety of pain tests have been employed in which various neuronal pathways are involved. It is possible that in one pathway opioids play a role whereas in another one an opioid component is absent (Watkins & Mayer, 1982).

The outcome of the hot plate test might be influenced by the sedative action of α-methyldopa. Such an action is well documented (Sjoerdsma, 1963; Clineschmidt *et al.*, 1980) and, therefore, the effects of α-methyldopa on the behaviour of rats in a small open field were investigated. Already 1 h after administration, α-methyldopa significantly decreased motor activity as shown by a lower locomotion score. The time-effect relationship of α-methyldopa-induced hypolocomotion is in agreement with an earlier observation (Clineschmidt *et al.*, 1980). Although locomotion is disturbed the first 3 h after the injection of α-methyldopa, the rats displayed a latency on the hot plate during this period not different from that of the saline-treated controls. Therefore, the sedative action of α-methyldopa appears to contribute little to the antinociceptive effect. Clineschmidt *et al.* (1980) demonstrated using α-adrenoceptor antagonists and FLA-63, a dopamine-β-hydroxylase inhibitor, that α-methyldopa reduces locomotor activity via the formation of α-methylnoradrenaline and subsequent stimulation of α_2-receptors. In contrast to its hypotensive effect, the hypolocomotive action of α-methyldopa is naltrexone-resistent. In addition, the hypothermia induced by α-methyldopa in conscious rats, that might play a role in the hypolocomotive mechanism of action of α-methyldopa, is also resistant to treatment with naltrexone (Sitsen & Nijkamp, 1984). Together, these results indicate that endogenous opioid peptides are not involved in the behavioural effects of α-methyldopa.

At present, the site(s) of action through which α-methyldopa induces analgesia and hypolocomotion are not known. α-Methylnoradrenaline acts on α_2-adrenoceptors, probably located in the NTS, to induce hypotension (Nijkamp & De Jong, 1975). Conway *et al.* (1979) demonstrated that in the NTS, but not in several other medullary and hypothalamic nuclei, after administration of 200 mg kg^{-1} of α-methyldopa to rats the accumulation and disappearance of α-methylnoradrenaline correlates with the time course of the hypotensive effect. The accumulation and disappearance of α-methyldopa and its metabolites, and the α-methyldopa-induced changes in the levels of endogenous neurotransmitters, might be factors which determine the time course of an effect of α-methyldopa. For example, the time-effect relationship of the antinociceptive effect of α-methyldopa, which is characterized by a 4 h lag period, could be determined by the above mentioned factors at the site of action.

Endogenous opioid substances are present in CSF (Kiser *et al.*, 1983; Jackson *et al.*, 1985). The concentration in CSF of pro-opiomelanocortin-derived peptides like β-endorphin appears to reflect release from the brain (Akil *et al.*, 1978; Hosobuchi *et al.*, 1979) and is not influenced by changes in the concentration of these peptides in plasma (Kalin *et al.*, 1987; Barna *et al.*, 1988). Administration of α-methyldopa to conscious rats had no effect on the concentration of β-ELIR in either plasma or CSF at various time points after drug administration. An increase in plasma β-ELIR has been implicated in the hypotensive mechanism of clonidine (Farsang *et al.*, 1984). However, in other studies hypotensive doses of clonidine did not affect (Levin *et al.*, 1986) or lowered (Yasunari *et al.*, 1985) the level of plasma β-ELIR. Therefore, a change in the concentration of β-ELIR in plasma appears not to be a step in the mechanism via which clonidine lowers blood pressure. This notion is supported by the present results showing no effect of a hypotensive dose of α-methyldopa on the level of β-ELIR in plasma. The lack of an effect of α-methyldopa on the CSF β-ELIR concentration might suggest that α-methyldopa does not induce release of β-endorphin in the brain *in vivo*. However, an α-methyldopa-induced increased release of β-endorphin in a restricted brain area, such as the NTS, which is not reflected by a change in CSF, may still occur.

In summary, α-methyldopa induces hypotension, antinociception and hypomotility without affecting the level of β-ELIR in either plasma or CSF. Naltrexone inhibited α-methyldopa-induced hypotension but not antinociception and hypomotility. Therefore, it is concluded that α-methyldopa induces hypotension, antinociception and hypomotility via (at least in part) different mechanisms. Endogenous opioid peptides play a role in the hypotensive action of α-methyldopa, whereas they appear not to be involved in the sedative and antinociceptive effects induced by α-methyldopa.

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