The pressor response to central administration of β -endorphin results from a centrally mediated increase in noradrenaline release and adrenaline secretion

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1 The effects of intracerebroventricular (i.c.v.) and intracisternal (i.c.) administration of β -endorphin (0.01, 0.1 and 1.0 nmol kg⁻¹) were examined in conscious rabbits.

2 After i.c.v. β -endorphin, mean arterial pressure (MAP) increased, heart rate (HR) fell, plasma noradrenaline, adrenaline and glucose increased and there was a rise in P_{aCO_2} and fall in P_{aO_2} ; these effects were reversed by intravenous (i.v.) naloxone (300 nmol kg⁻¹).

3 A combination of prazosin (2 mg kg⁻¹) and yohimbine (1 mg kg⁻¹), given i.v., prevented the rise in MAP induced by i.c.v. β -endorphin.

4 After i.c. β -endorphin, MAP, HR and plasma catecholamines were not significantly altered but there was a similar degree of respiratory depression.

5 Clonidine (1.0 μ g kg⁻¹, i.c.) reduced MAP and HR; these effects were not blocked by i.v. naloxone (6 μ mol kg⁻¹).

6 These results demonstrate that β -endorphin acts centrally, probably mainly on periventricular μ -opioid receptors, to increase adrenaline secretion and sympathetic nerve activity leading to α -adrenoceptor-mediated vasoconstriction. The respiratory depression is probably mediated by brainstem μ -receptors.

7 A role for β -endorphin in the central hypotensive action of α_2 -adrenoceptor agonists was opposed by finding that opioid receptor antagonism with naloxone did not block the effects of clonidine.

Introduction

β -Endorphin is a 31 amino acid peptide corresponding to residues 61–91 of β -lipotrophin, which is derived from proopiomelanocortin. It has been located in sites involved in central cardiovascular control such as the hypothalamus and nucleus of the tractus solitarius (NTS) (Akil *et al.*, 1984), and administration of β -endorphin either intravenously (i.v.), intracisternally (i.c.) or by microinjection into the NTS has been shown to reduce blood pressure in the rat (Lemaire *et al.*, 1978; Bolme *et al.*, 1978; Petty *et al.*, 1982). However, these studies were conducted on anaesthetized animals in which the pressor effects of opiates are reversed or abolished (Holaday 1982; May *et al.*, 1988). Central administration of selective μ - and δ -opioid agonists increases blood pressure in conscious rabbits (May *et al.*, 1989a), and therefore β -endorphin may be expected to raise blood pressure in conscious animals as it binds with equal affinity to μ - and δ -receptors (Paterson *et al.*, 1983).

We have studied the cardiovascular and respiratory effects of β -endorphin injected intracerebroventricularly (i.c.v.) or i.c. in conscious rabbits. The opioid receptor antagonist naloxone was used to confirm that these effects depended on stimulation of opioid receptors. The role of the nervous system was evaluated by measuring plasma catecholamines and by examining the effect of α -adrenoceptor blockade. There is evidence that β -endorphin mediates the hypotensive effect of clonidine (Farsang *et al.*, 1980; Ramirez-Gonzalez *et al.*, 1983) but this is controversial and has not been supported by other reports (Conway *et al.*, 1984; Head & de Jong, 1985). We have, therefore, examined the effect of opioid receptor antagonism with naloxone on the hypotensive effect of i.c. clonidine in conscious rabbits.

Methods

Surgical procedures

Experiments were carried out on normotensive, male Sandy Half-Lop rabbits (National Institute for Medical Research, Mill Hill, London) weighing between 2.6 and 3.4 kg, and allowed free access to food and water.

Injectations into the right lateral ventricle were made via cannulae (Harvard Apparatus) implanted aseptically under general anaesthesia using alphaxalone 0.9%/alphadolone acetate 0.3% (Saffan, Glaxovet) (1 ml i.v. followed by 0.25 ml i.v. as required). Coordinates were 1.0 mm caudal to bregma, 3.0 mm lateral to midline and 8.5 mm below the dura (May *et al.*, 1989c). β -Endorphin was injected i.c.v. in a volume of 25 μ l from a sterilised Hamilton syringe with a needle that protruded 1 mm from the cannula tip. The injection was flushed in with 25 μ l of saline from a needle that reached half way down the cannula shaft. For injection into the cisterna magna the catheter was inserted under Saffan general anaesthesia as previously described (May *et al.*, 1989b). Injectations were given in 25 μ l and flushed in with 20 μ l of saline; the dead volume of the catheter was 10 μ l. The injection sites were confirmed at the end of a series of experiments by injection of bromophenol blue (1%) either i.c.v. or i.c. followed by the appropriate volume of saline.

Experimental protocol

Animals were not used for at least 7 days post operation. Before each experiment cannulae were inserted into the central artery and marginal vein of the ear under 1% lignocaine local anaesthesia. The arterial cannula was connected to a pressure transducer (Bell and Howell) and BP and HR were recorded on a Devices polygraph.

Before the experiment the patency of the i.c.v. cannula was checked by allowing saline to flow into it under gravity. The

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experiment was continued only if saline flowed freely into the lateral ventricle and animals which showed any cardiovascular effects (usually bradycardia) were not used. This occurred rarely (<4%), when the animal had been used several times, and was associated with an infection around the cannula tip. No such problems were encountered with the i.c. cannulae. Different groups of rabbits were used for each experiment, except for one rabbit which was used in two groups and in this case the two experiments were 7 days apart.

β -Endorphin (human) (Cambridge Research Biochemicals) was dissolved in sterile, non-pyrogenic, isotonic saline and filtered through a sterile $0.2\mu\text{m}$ filter before injection. In the dose-response studies, two groups of six rabbits with either an i.c.v. or an i.c. cannula were studied while conscious. After a 30 min control period increasing doses of β -endorphin (0.01 , 0.1 and 1.0 nmol kg^{-1}) were given at hourly intervals. In previous studies we demonstrated that neither i.c.v. nor i.c. saline has any effect on MAP, HR, plasma catecholamines, respiration or behaviour in conscious rabbits (May *et al.*, 1989b).

The effect of naloxone hydrochloride (Sigma) on the responses to β -endorphin was examined in six conscious rabbits. β -Endorphin (1.0 nmol kg^{-1}) was given i.c.v., followed after 15 min by an i.v. dose of naloxone of 30 nmol kg^{-1} and after a further 20 min by a higher dose of naloxone (300 nmol kg^{-1}). Naloxone was dissolved in saline and given in a volume of $100\mu\text{L kg}^{-1}$. We have demonstrated that i.v. naloxone has no cardiovascular effects in conscious rabbits (May *et al.*, 1988).

Blockade of α_1 - and α_2 -adrenoceptors was produced with a combination of prazosin hydrochloride (Sigma) and yohimbine hydrochloride (Sigma). Prazosin hydrochloride (2 mg kg^{-1} in $400\mu\text{L kg}^{-1}$ of 0.04 M lactic acid) and yohimbine hydrochloride (1 mg kg^{-1} in $200\mu\text{L kg}^{-1}$ of distilled water) were given i.v. 10 min before i.c.v. administration of β -endorphin (1.0 nmol kg^{-1}). These doses of prazosin and yohimbine blocked the pressor effect of an i.v. dose ($5\mu\text{g kg}^{-1}$) of noradrenaline bitartrate (Sigma). After these doses the pressor effect of noradrenaline remained blocked for at least 1 h. A combination of α_1 - and α_2 -adrenoceptor antagonists was used as prazosin, given alone in doses from 2 to 5 mg kg^{-1} , only reduced the pressor effect of noradrenaline by a maximum of 80%.

The ability of endogenous opioid peptides to mediate the cardiovascular effects of clonidine was studied in six rabbits each with an indwelling i.c. cannula. The effect of a near maximal dose of i.c. clonidine ($1\mu\text{g kg}^{-1}$) was examined after pretreatment with either i.v. saline ($333\mu\text{L kg}^{-1}$) or i.v. naloxone ($6\mu\text{mol kg}^{-1}$) given 10 min before clonidine; the treatments were randomised.

Blood collection and analysis

Catecholamines were measured in plasma from blood (2.5 mL) collected from the arterial cannula into cooled tubes containing $50\mu\text{L}$ EGTA (0.095%)/glutathione (0.06%) and kept on ice until centrifugation at 4°C and then storage at -70°C . Catecholamines were separated by high performance liquid chromatography (h.p.l.c.) and detected by electrochemical detection (May *et al.*, 1988). Plasma glucose was measured by a glucose oxidase method on plasma from arterial blood (2.0 mL) collected into fluoride oxalate tubes. Immediately following blood collection an equal volume of isotonic saline was given i.v. Arterial blood ($100\mu\text{L}$) was collected into a heparinised capillary tube for the measurement of blood gases (Instrument Laboratory 230 blood gas analyser).

Statistical analysis

Data are presented as means $\pm$ s.e.mean. Analysis was performed on the differences from the control values by the SAS statistical programme. The control values for MAP and HR consist of the mean of the six readings taken during the control period. A logarithmic transformation was performed on the values for plasma noradrenaline, adrenaline and

glucose before analysis. All the cardiovascular and biochemical data were subjected to analysis of variance and where the null hypothesis was rejected Bonferroni's comparisons were performed on the means; $P < 0.05$ was considered significant.

Results

Effects of i.c.v. and i.c. β -endorphin

β -Endorphin given i.c.v. caused a dose-related increase in MAP in conscious rabbits (Figure 1). The lowest dose (0.01 nmol kg^{-1}) had no effect but after the intermediate dose (0.1 nmol kg^{-1}) MAP reached $11 \pm 5\text{ mmHg}$ above control. After the highest i.c.v. dose (1.0 nmol kg^{-1}), MAP was significantly elevated at all times ($P < 0.05$) and reached $26 \pm 6\text{ mmHg}$ above control after 30 min. There was an initial fall in HR but by 60 min it had increased above control. After i.c. β -endorphin there were no changes after the two lowest doses. After the highest dose (1.0 nmol kg^{-1}) MAP reached a maximum of $12 \pm 6\text{ mmHg}$ above control after 60 min but this was not significant.

Samples for plasma catecholamines were taken at the end of the control period, immediately before the highest dose (60 min after the middle dose), and at 5, 30 and 60 min after the highest dose. Following i.c.v. administration of the highest

Figure 1 Effect of increasing doses of β -endorphin (0.01 , 0.1 and 1.0 nmol kg^{-1}) given at 60 min intervals either i.c.v. (●) or i.c. (□) on mean arterial pressure (MAP), heart rate (HR), plasma noradrenaline and adrenaline in conscious rabbits. The control values for MAP and HR are the mean of six readings taken during the control period. Each point represents the mean ($n = 6$), with s.e.mean shown by vertical bars.

Figure 2 Effect of increasing doses of β -endorphin (0.01 , 0.1 and 1.0 nmol kg^{-1}) given at 60 min intervals either i.c.v. ($\bullet$) or i.c. ($\square$) on respiration rate (RR), PaCO_2 and PaO_2 in conscious rabbits. Each point represents the mean ($n = 6$), with s.e.mean shown by vertical bars.

dose of β -endorphin, plasma noradrenaline was not increased after 5 min but was significantly elevated after 30 and 60 min ($P < 0.05$) (Figure 1). This dose of β -endorphin caused an immediate, significant increase in plasma adrenaline after 5 min and it remained elevated after 30 and 60 min ($P < 0.05$) (Figure 1). After i.c. β -endorphin (1.0 nmol kg^{-1}) there was a smaller rise in noradrenaline than after i.c.v. administration and no change in adrenaline (Figure 1). Plasma glucose significantly increased from $7.25 \pm 0.17 \text{ mmol l}^{-1}$ to 11.78 ± 0.94 and $10.56 \pm 0.73 \text{ mmol l}^{-1}$ at 30 and 60 min respectively after i.c.v. β -endorphin (1.0 nmol kg^{-1}). After i.c. administration the increases were smaller but remained significant; plasma glucose increased from $7.09 \pm 0.15 \text{ mmol l}^{-1}$ to 8.72 ± 0.43 and $8.91 \pm 0.33 \text{ mmol l}^{-1}$ at 30 and 60 min respectively.

Blood gases were not significantly changed after the two lowest doses of β -endorphin given either i.c.v. or i.c. After the highest i.c.v. dose there was a gradual increase in PaCO_2 and a fall in PaO_2 , and these changes were significant after 30 and 60 min ($P < 0.05$) (Figure 2). Following i.c. administration there were similar changes in PaCO_2 and PaO_2 (Figure 2). There were no changes in respiration rate after either i.c.v. or i.c. β -endorphin.

β -Endorphin also caused sedation after i.c.v. administration. There was no effect after the lowest dose but after 0.1 nmol kg^{-1} the rabbits were less active and after 1.0 nmol kg^{-1} they became heavily sedated. No behavioural changes were observed after i.c. administration.

Effect of i.v. naloxone on the responses to i.c.v. β -endorphin

In six rabbits i.c.v. β -endorphin (1.0 nmol kg^{-1}) increased MAP ($P < 0.05$) and reduced HR after 15 min (Figure 3). After 10 min plasma noradrenaline and adrenaline had significantly increased ($P < 0.05$), PaCO_2 had increased by $6.5 \pm 1.8 \text{ mmHg}$ ($P < 0.05$) and PaO_2 had fallen by $12.8 \pm 2.6 \text{ mmHg}$ ($P < 0.05$). Naloxone (30 nmol kg^{-1}) given i.v. 15 min after β -

Figure 3 Effect of i.v. naloxone on the changes in mean arterial pressure (MAP), heart rate (HR) and plasma catecholamines in response to i.c.v. β -endorphin (1.0 nmol kg^{-1}) in conscious rabbits. Naloxone (30 and 300 nmol kg^{-1}) was given 15 and 35 min respectively after β -endorphin. The control values for MAP and HR are the mean of six readings taken during the control period. Each point represents the mean ($n = 6$), with s.e.mean shown by vertical bars.

endorphin caused a transient reduction in MAP, returned HR to control and reduced plasma catecholamine levels (Figure 3). However, PaCO_2 continued to increase to reach $12.2 \pm 1.1 \text{ mmHg}$ above control and PaO_2 to fall to reach $18.9 \pm 3.0 \text{ mmHg}$ below control ($P < 0.05$). Subsequent administration of a higher dose of naloxone (300 nmol kg^{-1}), given 35 min after β -endorphin (20 min after the lower dose of naloxone), caused MAP to return to and remain at control levels, and HR to increase to $48 \pm 14 \text{ beats min}^{-1}$ above control. After this dose of naloxone plasma catecholamines returned towards control (Figure 3). The higher dose of naloxone abolished the β -endorphin-induced respiratory depression and reversed its sedative effect.

Effect of α -adrenoceptor blockade on the responses to i.c.v. β -endorphin

A combination of prazosin (2 mg kg^{-1}) and yohimbine (1 mg kg^{-1}), given i.v., significantly reduced MAP and increased HR within 5 min in six rabbits (Figure 4). After 10 min there were significant increases in plasma noradrenaline and plasma adrenaline ($P < 0.05$). Subsequent administration of β -endorphin (1.0 nmol kg^{-1}) caused no increase in MAP or fall in HR, although there were further increases

Figure 4 Effect of i.v. prazosin (2 mg kg^{-1}) and yohimbine (1 mg kg^{-1}) on the changes in mean arterial pressure (MAP), heart rate (HR) and plasma catecholamines in response to i.c.v. β -endorphin (1.0 nmol kg^{-1}) in conscious rabbits. Prazosin and yohimbine were given together 10 min before β -endorphin. The control values for MAP and HR are the mean of six readings taken during the control period. Each point represents the mean ($n = 6$), with s.e.mean shown by vertical bars.

above control in noradrenaline and plasma adrenaline within 5 min. Catecholamines remained elevated at this level for the remainder of the experiment (Figure 4).

α -Adrenoceptor blockade did not alter the β -endorphin-induced respiratory depression. At 60 min after β -endorphin respiration rate was unchanged, P_{aCO_2} was increased by $19.1 \pm 3.0\text{ mmHg}$ ($P < 0.05$) and P_{aO_2} was reduced by $16.0 \pm 2.7\text{ mmHg}$ ($P < 0.05$). Administration of i.v. naloxone (300 nmol kg^{-1}) returned blood gases to control levels within 5 min.

Effect of opioid receptor antagonism on the response to i.c. clonidine

Clonidine ($1.0\text{ }\mu\text{g kg}^{-1}$) given i.c., 10 min after i.v. saline ($333\text{ }\mu\text{l kg}^{-1}$), reduced MAP by $19 \pm 5\text{ mmHg}$ ($P < 0.05$) after 15 min and HR by $38 \pm 10\text{ beats min}^{-1}$ ($P < 0.05$) after 10 min (Figure 5). There was a tendency for plasma noradrenaline to fall after clonidine but this did not reach significance and there was no effect on plasma adrenaline. At 60 min after clonidine, administration of i.v. naloxone ($6\text{ }\mu\text{mol kg}^{-1}$) caused no

Figure 5 Effect of i.c. clonidine ($1.0\text{ }\mu\text{g kg}^{-1}$) on mean arterial pressure (MAP), heart rate (HR) and plasma catecholamines after pretreatment with i.v. saline ($333\text{ }\mu\text{l kg}^{-1}$) (●) or i.v. naloxone ($6\text{ }\mu\text{mol kg}^{-1}$) (□). In the saline treated group i.v. naloxone ($6\text{ }\mu\text{mol kg}^{-1}$) was given 60 min after clonidine (○). The control values for MAP and HR are the mean of six readings taken during the control period. Each point represents the mean ($n = 6$), with s.e.mean shown by vertical bars.

change in MAP or HR. When i.c. clonidine was given 10 min after i.v. naloxone ($6\text{ }\mu\text{mol kg}^{-1}$), the clonidine-induced changes in MAP, HR and plasma noradrenaline were not significantly different from those when it was given after saline (Figure 5).

Discussion

This study demonstrates that in conscious rabbits, β -endorphin acts centrally to cause a dose-related increase in MAP, respiratory depression and sedation. The cardiovascular effects do not result from leakage of β -endorphin to the periphery as i.v. β -endorphin (29 nmol kg^{-1}) does not increase MAP in conscious rabbits of the same strain used in the present experiments (personal communication, Dr S.J. Potocnik). Following i.c.v. β -endorphin the rise in pressure was accompanied by a large increase in plasma adrenaline and a smaller rise in plasma noradrenaline. In the rabbit, noradrenaline is not found in the adrenal medulla (Hokfelt, 1952) and following splanchnic nerve stimulation only adrenaline is secreted (West, 1950), indicating that β -endorphin increases sympathetic outflow to both noradrenergic nerves and to the adrenal medulla. All the effects of β -endorphin were reversed

by naloxone demonstrating that they depended on stimulation of opioid receptors. This is not the result of an opposing hypotensive effect of naloxone as, in agreement with other authors (Petty & Reid, 1982; Rutter *et al.*, 1987), we have demonstrated that i.v. naloxone given alone has no effect on MAP or HR in conscious rabbits (May *et al.*, 1988). The lower dose of naloxone briefly returned MAP and HR to control levels, and then as MAP increased there was a corresponding fall in HR. After the higher dose of naloxone, when the rise in MAP was permanently reversed, HR increased above control. Similar increases in HR occurred when naloxone was given after the μ -receptor agonists DAMGO (Pfeiffer *et al.*, 1983; May *et al.*, 1989a) and morphine (May *et al.*, 1989b), and may partly result from antagonism by naloxone of opioid-induced vagal stimulation as the increase in HR is reduced by pretreatment with atropine methyl nitrate (Pfeiffer *et al.*, 1983).

The β -endorphin-induced rise in MAP was inhibited by a combination of the selective α_1 - and α_2 -adrenoceptor antagonists, prazosin and yohimbine respectively, demonstrating that the pressor effect was due to the increase in sympatho-adrenal outflow leading to α -adrenoceptor-mediated vasoconstriction. The requirement for both α -adrenoceptor antagonists to block the pressor effects of noradrenaline indicates that in the rabbit, noradrenaline-induced vasoconstriction is mediated by postsynaptic α_2 - as well as α_1 -adrenoceptors.

After i.c. β -endorphin the changes in MAP and noradrenaline were considerably less and there was no rise in adrenaline but the degree of respiratory depression was similar to that after i.c.v. administration. The responses to β -endorphin did not result from non-specific effects due to injection of the vehicle as in conscious rabbits, i.c.v. and i.c. saline have no effect on the measured variables (May *et al.*, 1989b).

In previous studies in conscious rabbits, using an identical protocol, we demonstrated that i.c.v. and i.c. injection of the selective μ -opioid agonist (D-Ala²-, MePhe⁴-, Gly⁵-ol)enkephalin (DAMGO) caused a similar pattern of responses to β -endorphin (May *et al.*, 1989a). In the same study the selective δ -opioid agonist (D-Pen^{2,5})enkephalin (DPDPE) increased MAP, but in contrast to DAMGO, administration of DPDPE increased HR, did not increase adrenaline secretion or cause respiratory depression and induced behavioural activation rather than sedation. Thus, although β -endorphin binds equally to μ - and δ -opioid receptors (Paterson *et al.*, 1983), its effects following i.c.v. and i.c. injection are qualitatively the same as those of a selective μ -agonist suggesting that they result predominantly from an action on μ -receptors.

The opioid receptors leading to the increase in sympathetic outflow are probably located mainly in periventricular structures as the responses are greater after i.c.v. than i.c. administration. We have demonstrated the presence of μ -opioid receptors in hypothalamic nuclei in rabbit brain by *in vitro* autoradiography (May *et al.*, 1989a), and this may be a site at which β -endorphin acts to increase sympatho-adrenal outflow. This is supported by the finding that in conscious rats microinjection of the μ -agonist DAMGO into the anterior hypothalamus produced similar increases in sympatho-adrenal outflow and blood pressure to those seen after i.c.v. administration (Pfeiffer *et al.*, 1983). The tendency for MAP to rise after i.c. administration indicated that additional actions at brainstem sites, such as the dorsal motor nucleus of the vagus which contains μ -receptors (May *et al.*, 1989a), cannot be discounted. More discrete administration of β -endorphin will be required to localise accurately the site, or sites, at which β -endorphin acts to increase sympatho-adrenal outflow.

Opioid-induced respiratory depression results from an action on μ - but not δ - or κ -receptors (May *et al.*, 1989a) located in the ventral medulla (Florez *et al.*, 1968). This area

will be reached by drugs given i.c.v. and i.c. and may be the site at which β -endorphin acts to produce respiratory depression as similar responses occurred after both routes of administration. The hyperglycaemia following i.c.v. β -endorphin is probably mainly due to increased adrenaline secretion, as has been shown following central administration of β -endorphin in conscious rats (Van Loon & Appel, 1981) and following i.v. administration of the μ -opioid agonist morphine in conscious rabbits (May *et al.*, 1988). The rise in plasma glucose after i.c. β -endorphin, when there was no increase in plasma adrenaline, could result from a mechanism mediated by central opioid receptors but independent of catecholamine release as has previously been demonstrated in conscious rabbits (Knudtson, 1986).

The pressor response to β -endorphin demonstrated in this study is at variance with other reports in which i.c. β -endorphin produced a temporary rise followed by a fall in blood pressure in anaesthetized rats (Bolme *et al.*, 1978), a fall in blood pressure and HR in anaesthetized cats (Feldberg & Wei, 1978), and microinjection into the NTS of anaesthetized rats reduced blood pressure and HR (Petty *et al.*, 1982). The absence of any increase in pressure in these studies may result from the use of anaesthetics, which have been shown to block or reverse the pressor effects resulting from stimulation of opioid receptors (Kayaalp & Kaymakcalon, 1966; Holaday, 1982; May *et al.*, 1988).

β -Endorphin has been proposed as a mediator of the centrally-induced hypotensive action of α_2 -adrenoceptor agonists (Farsang *et al.*, 1980; Ramirez-Gonzalez, 1983; Marmo *et al.*, 1984; Tackett & Laskey, 1987). However, this has not been confirmed by other reports (Conway *et al.*, 1984; Head & de Jong, 1985) and our finding that β -endorphin increases blood pressure does not support this hypothesis. In view of these differences we examined the effect of opioid receptor antagonism on the response to clonidine in conscious rabbits. Clonidine was given i.c. to avoid any peripheral effects; the dose of naloxone was the same as that used in the majority of studies and the animals were conscious to exclude the effects of anaesthetics. In our studies naloxone did not inhibit the clonidine-induced fall in blood pressure, heart rate and plasma catecholamines, or reverse the hypotension when given after clonidine. These results indicate that in conscious, normotensive rabbits endogenous opioid peptides are not involved in the central hypotensive effect of clonidine. This does not exclude a role for opioid peptides in the hypotensive action of clonidine in hypertensive animals, as has been suggested in rats (Farsang *et al.*, 1980). However, this finding has not been confirmed by other authors in conscious or anaesthetized, spontaneously hypertensive and normotensive rats (Conway *et al.*, 1984; Head & de Jong, 1985). It has been suggested that the inter-relationship described in some reports may result from the use of anaesthetics or the effect of stress induced by indirect methods of measuring blood pressure (Head & de Jong, 1985), and our present findings in conscious, unstressed animals support this hypothesis.

In conclusion, we have demonstrated that in conscious rabbits, β -endorphin acts on central opioid receptors, possibly in the hypothalamus, to increase sympatho-adrenal outflow and elevate blood pressure. Endogenous opioid peptides such as β -endorphin may mediate the cardiovascular responses to stress as opioid receptor antagonists prevent stress-induced increases in blood pressure (Naranjo *et al.*, 1986). The inability of naloxone to prevent the clonidine-induced fall in MAP and HR indicates that endogenous opioid peptides do not mediate the central hypotensive effects of α_2 -adrenoceptor agonists.

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