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Circulating [Met]enkephalin and catecholamine responses to acute hypotension and hypertension in anaesthetized greyhounds

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1 The effects of either hypotension induced by sodium nitroprusside or hexamethonium or hypertension produced by angiotensin II or noradrenaline on the circulating levels of methionine enkephalin ([Met]enkephalin)-like immunoreactivity (MLI), adrenaline and noradrenaline in anaesthetized greyhounds were examined.

2 Nitroprusside infusions (200 and 400 $\mu g\,min^{-1}$) induced a fall in blood pressure accompanied by significant rises in plasma MLI and catecholamine concentrations.

3 Concomitant administration of a high dose of naloxone did not alter the fall in blood pressure produced by nitroprusside but was associated with greater rises in circulating MLI and catecholamines when compared to nitroprusside alone, suggesting that [Met]enkephalin is not involved in the hypotensive action of nitroprusside.

4 Intravenous hexamethonium ($2.5\,mg\,kg^{-1}$) provoked a fall in blood pressure which was not associated with any changes in plasma MLI. However, it produced a fall in plasma noradrenaline and a rise in plasma adrenaline. Thus it appears that neural mechanisms are required, at least in part, for the release of MLI.

5 Angiotensin II ($1.25\,\mu g\,kg^{-1}\,min^{-1}$) and noradrenaline ($8\,\mu g\,kg^{-1}\,min^{-1}$) infusions produced an elevation in blood pressure without altering the circulating MLI levels.

6 Study of the molecular forms of circulating MLI, before and during hypotension, revealed that the large molecular weight enkephalin-containing peptides with approximate molecular sizes of 18kD and 8kD were the predominant forms both in the basal and stimulated states.

7 It is concluded that circulating [Met]enkephalin is not involved in the tonic control of blood pressure but it may modulate catecholamine release following hypotension as part of the stress response.

Introduction

There is considerable evidence at present for the involvement of the endogenous opioid peptides in the control of cardiovascular function. The administration of opiates and opioid peptides provokes profound cardiovascular changes including marked hypotension in animals and man (Fennessy & Rattray, 1971; Laubie *et al.*, 1977; Florez & Mediavilla, 1977; Lemaire *et al.*, 1978). Moreover areas in the brain known to play a role in the autonomic control of cardiovascular function are rich in opioid receptors and opioid peptides (Atweh & Kumar, 1977; Wamsley *et al.*, 1982). The cardiovascular role of opioids has further been suggested by the efficacy of naloxone in reversing the fall in blood pressure and improving cardiac indices and survival in various forms of circulatory shock demonstrated both in animals (Holaday & Faden, 1978) and man (Peters *et al.*, 1981; Hughes, 1984). In addition it has been shown that plasma concentrations of immunoreactive methionine-enkephalin ([Met]enkephalin)-like peptides rise during either endotoxin (Evans *et al.*, 1984) or haemorrhagic (Lang *et al.*, 1982) shock in dogs. However the mechanisms of action of opioids in inducing cardiovascular changes are not yet fully elucidated. In general the effects of administered Met-enkephalin and analogues on the cardiovascular sys-

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tem, especially blood pressure, depend on the animal species studied, route of administration and anaesthesia used thus causing an increase in blood pressure in conscious animals (Schaz *et al.*, 1980; Yukimura *et al.*, 1981; Sander & Giles, 1982) or a decrease in blood pressure in anaesthetized animals (Wei *et al.*, 1980; Eulie & Rhee, 1984). When tested, these effects are blocked by naloxone suggesting the involvement of opioid receptors. The site(s) of action of circulating enkephalins on the cardiovascular system is currently under extensive study. The heart contains opioid receptors (Burnie, 1981) and the enkephalins have been shown to have a direct positive inotropic effect on cultured cardiac myocytes (Laurent *et al.*, 1985). However, Saunders & Thornhill (1985) showed no inotropic action of the enkephalins on rat isolated atria. Other peripheral sites of action of the enkephalins include the sympathetic nerves (Gaddis & Dixon, 1982), peripheral vasculature (Sander *et al.*, 1981), adrenal chromaffin cells (Kumakura *et al.*, 1980), baroreceptor reflex activity (Rubin, 1984) or through pulmonary 'J' chemoreceptors (Sapru *et al.*, 1981). Centrally, circulating enkephalins could act on the brain opioid receptors adjacent to the circumventricular regions, areas known to have an incomplete blood-brain barrier (Holaday, 1983; Eulie & Rhee, 1984). Therefore it was of interest to us to study the circulating levels of [Met]enkephalin-like immunoreactivity (MLI) in response to cardiovascular changes. In the present series of experiments we studied, in anaesthetized greyhounds, the responses of plasma MLI, adrenaline and noradrenaline to: (a) acute hypotension induced by an infusion of nitroprusside; with and without concomitant naloxone infusion, (b) acute hypotension induced by intravenous hexamethonium and (c) hypertension induced by angiotensin II and noradrenaline infusions. In addition, we characterized the molecular forms of circulating MLI by means of gel-filtration chromatography.

Methods

Animals and surgical procedures

Anaesthetized greyhounds with an average body weight of 30 kg were used in these experiments which allowed us to withdraw sufficient blood for the required assays without interfering with the circulating levels of MLI (Medbak *et al.*, 1983). After an overnight fast, anaesthesia was induced by intravenous methohexitone sodium (12.5 mg kg^{-1}) through a forelimb vein with the overlying skin locally anaesthetized. This vein was subsequently used for the administration of various drugs. Anaesthesia was maintained by a chloralose-urethane mixture (50 and 500 mg kg^{-1} respectively) followed by regular supplements. The trachea was then cannulated and voluntary (spontaneous) respiration continued using room air throughout the experiment. The right jugular vein was cannulated for blood sampling and the left carotid artery was catheterized for the continuous measurements of blood pressure and heart rate.

A control period of at least 30 min was allowed before each drug administration.

Blood sampling

Venous blood was withdrawn for the measurements of plasma MLI, adrenaline and noradrenaline before, during and, at regular intervals after each drug administration. The first 2 ml of blood of each sample was discarded. Previous experiments had shown that the concentrations of MLI in venous and arterial blood were not significantly different.

Protocol

In a group of five dogs, sodium nitroprusside (200 $\mu g\ min^{-1}$) was infused for 10 min.

In another group of greyhounds, a randomised within subject comparison of sodium nitroprusside (400 $\mu g\ min^{-1}$) infused for 10 min with and without concomitant naloxone infusion was carried out with an interval of at least 1 h between the two treatments. Naloxone (2.4 mg intravenously as a bolus dose followed by a constant infusion at a rate of 0.05 mg min^{-1}) was started 10 min before nitroprusside and continued for another 10 min after cessation of nitroprusside infusion ($n = 4$).

In three of these animals and after a 60 min rest period the effect of hypertension on plasma MLI and adrenaline was studied using noradrenaline (8 $\mu g\ kg^{-1}\ min^{-1}$) infused for 10 min.

In a further group of four dogs angiotensin II (1.25 $\mu g\ kg^{-1}\ min^{-1}$) was infused for 10 min followed by sodium nitroprusside (400 $\mu g\ min^{-1}$) for 10 min followed by intravenous hexamethonium (2.5 mg kg^{-1}). Each animal received all three drugs and in that order with a 90 min rest period in between the drug administrations.

Drugs

Sodium nitroprusside was obtained from Roche, naloxone from Du Pont Pharmaceuticals, angiotensin II from Ciba, hexamethonium from Sigma, and noradrenaline from Winthrop Laboratories.

Radioimmunoassay for plasma MLI

Blood samples (10 ml) were withdrawn from the right jugular vein and transferred into lithium-heparin tubes containing 500 μl (10,000 kallikrein inactivator

units) of aprotinin proteinase inhibitor (Trasylol, Bayer). These were spun at 2 *g* for 10 min and the supernatant plasma was pipetted into plastic tubes containing 750 μl of 20 mmol l^{-1} glycine in 1 mol l^{-1} HCl and immediately frozen by contact with solid CO_2 pellets. All samples were stored at −20°C until assayed. MLI was measured using an extracted and highly sensitive C-terminally directed radioimmunoassay which will detect Met-enkephalin and some of its larger molecular forms (Clement-Jones *et al.*, 1980).

Measurement of plasma catecholamines

Plasma adrenaline and noradrenaline were measured by the method described by Bouloux *et al.* (1985). Briefly, 10 ml blood was collected and transferred into lithium-heparin tubes containing sodium metabisulphite (1 mmol l^{-1} final concentration). The tubes were centrifuged at 2 *g* for 10 min and plasma was transferred into plastic tubes and flash-frozen using solid CO_2 pellets. Samples were stored at −70°C until assayed. Catecholamines were extracted from plasma using alumina acid type WA-4 (Sigma) and assayed by high performance liquid chromatography and electrochemical detection.

Gel filtration chromatography

Gel filtration chromatography of plasma samples to characterize the molecular forms of circulating MLI was carried out on a Sephadex G75 column run in acid dissociating conditions (1% formic acid). Column fractions were dried down, reconstituted and assayed following treatment with trypsin and carboxypeptidase B, a procedure used to detect internal enkephalin sequences (Medbak *et al.*, 1983).

All results are expressed as mean ± s.e.mean and statistical analysis was done using Student's *t* test. Part of this work was communicated to the British Pharmacological Society Meeting at the University of Southampton, 17–19 July, 1985.

Results

Following the infusion of sodium nitroprusside (200 μg min^{-1}) the blood pressure fell significantly with the systolic falling from a basal of 183 ± 17 mmHg to 116 ± 14 mmHg and the diastolic from 140 ± 16 mmHg to 87 ± 13 mmHg at 10 min. This was associated with significant rises in plasma MLI from a basal of 36 ± 6 pg ml^{-1} to a peak of 79 ± 11 pg ml^{-1} after 10 min ($P < 0.005$), plasma adrenaline from a basal of 2.80 ± 0.43 nmol l^{-1} to a peak of 4.74 ± 0.45 nmol l^{-1} after 5 min ($P < 0.01$) and plasma noradrenaline from a basal of 2.34 ± 0.54 nmol l^{-1} to a peak of 4.35 ± 0.34 nmol l^{-1} after 10 min ($P < 0.001$; Figure 1). The peak rises in plasma MLI and catecholamines coincided with the fall in blood pressure. When sodium nitroprusside administration was stopped the blood pressure, plasma MLI and catecholamine levels returned to their baseline values within 10–20 min. In the next group of greyhounds the effects of naloxone on the blood pressure, plasma MLI and catecholamine responses to sodium nitroprusside infusion were studied. Naloxone did not alter the basal blood pressure readings and had no effect on the basal levels of plasma MLI, adrenaline and noradrenaline. Following sodium nitroprusside infusion (400 μg min^{-1}) there were significant decreases in both the systolic (216 ± 13 mmHg falling to 148 ± 17 mmHg after 10 min) and diastolic (189 ± 11 to 123 ± 17 mmHg) blood pressures which were unchanged in the presence of naloxone (systolic 220 ± 8 to 158 ± 5; diastolic 181 ± 8 to 133 ± 6 mmHg). However, as shown in Figure 2 the rises in plasma MLI (after 10 min), adrenaline (after 5 and 10 min) and noradrenaline

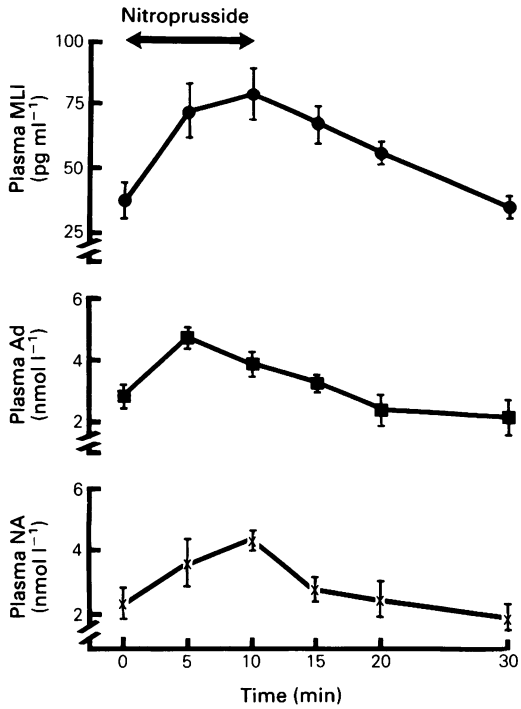

Figure 1 Plasma [Met]enkephalin-like immunoreactivity (MLI) (●), adrenaline (Ad) (■) and noradrenaline (NA) (×) levels in five anaesthetized greyhounds in response to sodium nitroprusside infusion (200 μg min^{-1}) (←→). Mean values are shown with s.e.mean indicated by vertical bars.

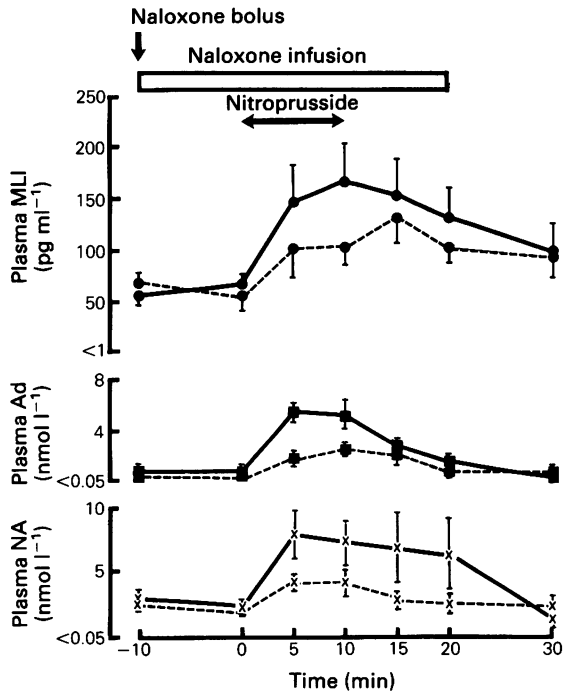

Figure 2 Plasma [Met]enkephalin-like immunoreactivity (MLI) (●), adrenaline (■) and noradrenaline (×) responses to sodium nitroprusside infusion (400 μg min^{-1}) (←→) in the presence (solid lines) and absence (interrupted lines) of naloxone (□) administered as a bolus dose (↓) followed by an infusion (see text) in four anaesthetized greyhounds; mean values are shown with s.e.mean indicated by vertical bars.

(after 5, 10 and 15 min) after sodium nitroprusside infusion were greater in the presence of naloxone ($P < 0.05$; paired t test).

Figure 3 shows the blood pressure, plasma MLI and catecholamine changes in response to the administration of angiotensin II, sodium nitroprusside and hexamethonium. The systolic and diastolic blood pressures rose significantly following angiotensin II infusion and decreased after both sodium nitroprusside infusion and intravenous hexamethonium. There were opposite changes in heart rate in each case. However, plasma MLI changed significantly only after sodium nitroprusside rising from a basal level of 55 ± 10 pg ml^{-1} to a peak of 179 ± 63 pg ml^{-1} after 15 min ($P < 0.001$). This MLI rise was associated with significant rises in plasma adrenaline from a basal level of 2.27 ± 1.12 nmol l^{-1} to a peak of 8.54 ± 2.70 nmol l^{-1} after 5 min ($P < 0.001$) and noradrenaline from a basal level of 3.71 ± 0.36 nmol l^{-1} to a peak of 6.45 ± 1.50 nmol l^{-1} after 5 min ($P < 0.001$). Furthermore, as shown in Figure 3, plasma adrenaline, unlike plasma MLI, rose slightly though significantly following both angiotensin infusion (from a basal of 0.49 ± 0.05 nmol l^{-1} to 2.05 ± 0.41 nmol l^{-1} after 5 min) and intravenous hexamethonium (from a basal of 0.50 ± 0.10 nmol l^{-1} to 1.02 ± 0.29 nmol l^{-1} after 5 min). Plasma noradrenaline however did not change following angiotensin but fell significantly after hexamethonium (from a basal of 2.93 ± 0.14 nmol l^{-1} to 2.05 ± 0.32 nmol l^{-1} after 5 min; $P < 0.05$).

In addition to angiotensin II administration, the effect of hypertension on plasma MLI and adrenaline was further studied using noradrenaline infusion. Figure 4 shows that following noradrenaline infusion the rise in blood pressure did not provoke changes in either circulating MLI or adrenaline levels.

In an attempt to characterize the molecular forms of circulating MLI, gel filtration chromatography of neat plasma was carried out on a sephadex G75 column. Chromatography of plasma obtained in the basal and stimulated states showed that the larger molecular forms of [Met]enkephalin with approximate molecular sizes of 18kD and 8kD were the predominant forms. However there were no marked differences in the chromatographic profiles between the basal and stimulated states (Figure 5).

Discussion

In this study we have shown that nitroprusside infusion in anaesthetized greyhounds led to hypotension which was associated with significant rises in plasma MLI, adrenaline and noradrenaline concentrations (Figure 1). The hormonal and blood pressure changes returned to baseline values shortly after the cessation of nitroprusside infusion. These results are in agreement with previous reports that during hypotension, induced by circulatory shock the circulating levels of MLI are elevated (Lang *et al.*, 1982; Evans *et al.*, 1984). We employed two different doses of nitroprusside (200 and 400 μg min^{-1}) in our experiments. The fall in blood pressure was the same with both doses. The basal plasma MLI concentrations were marginally different in the 2 groups; however, in both the levels were within the normal range (found in a series of studies in our laboratories) and both returned to baseline concentrations following stimulation. However, the peak rises in plasma MLI and adrenaline but not noradrenaline following the high dose were greater compared to the lower dose (120% rise in MLI after lower dose versus 225% following higher dose; 69% versus 276% for adrenaline and 86% versus 74% for noradrenaline, Figures 1 and 3). The reason for the apparent dissociation between the falls in blood pressure and the rises in the

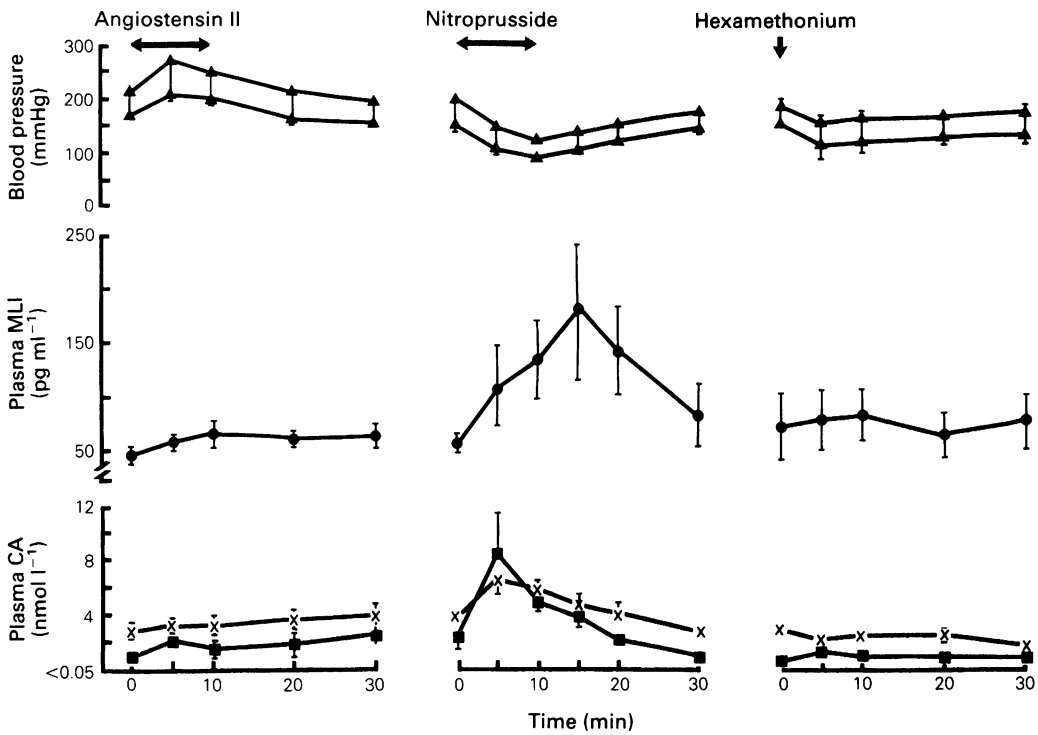

Figure 3 Blood pressure (▲), plasma [Met]enkephalin-like immunoreactivity (MLI) (●), adrenaline (■) and noradrenaline (×) responses to angiotensin II (1.25 μg kg^{-1} min^{-1}), sodium nitroprusside (400 μg min^{-1}) and hexamethonium (2.5 mg kg^{-1}) administration in the same group of anaesthetized greyhounds; mean values are shown with s.e.mean indicated by vertical bars ($n = 4$).

circulating hormone levels is not clear at present.

[Met]-enkephalin has been shown to produce hypotension in animals (Moore & Dowling, 1980) and Hanbauer *et al.* (1982) have demonstrated that in reserpine-treated dogs with catecholamine depletion, splanchnic nerve stimulation produced an increase in the release of adrenal enkephalins with a fall in blood pressure rather than the usual rise. Moreover Eulie & Rhee (1984) showed that in anaesthetized rabbits, [Met]enkephalin potentiated the hypotension induced by nitroprusside. The possibility then arises that endogenously produced [Met]enkephalin could contribute to the hypotensive effect of nitroprusside. However our data, using naloxone, do not support this hypothesis. A within subject comparison using nitroprusside with and without concomitant high dose naloxone infusion showed that the fall in blood pressure was similar in the two experiments. This argues against opioid mechanisms being a major contributory factor in the hypotensive action of nitroprusside. Moreover these results differ from those in shock (Holaday & Faden, 1978) and haemorrhagic hypotension (Schadt & Gaddis, 1985) where naloxone was shown to be effective in reversing the fall in blood pressure. The rises in plasma adrenaline and noradrenaline following nitroprusside-induced hypotension were greater with naloxone than in the control experiment (Figure 2) suggesting that naloxone removes the opioid receptor-mediated inhibitory effect of [Met]enkephalin on the sympathoadrenomedullary activity (Kumakura *et al.*, 1980). However the function of the greater rise in plasma MLI in the presence of naloxone is not clear at present. It could represent a consequence of the greater rise in plasma catecholamines thus acting as a neuromodulator at adrenomedullary or neural sites to control the release of catecholamines (Costa *et al.*, 1983).

The source(s) of plasma MLI and the mechanisms involved in its release are not fully clear at present. [Met]enkephalin-containing peptides exist in high concentrations in the adrenal medualla (Kilpatrick *et al.*, 1982) co-stored with catecholamines (Viveros *et al.*, 1979) from where they can be released into the circulation. Other possible sources of circulating MLI

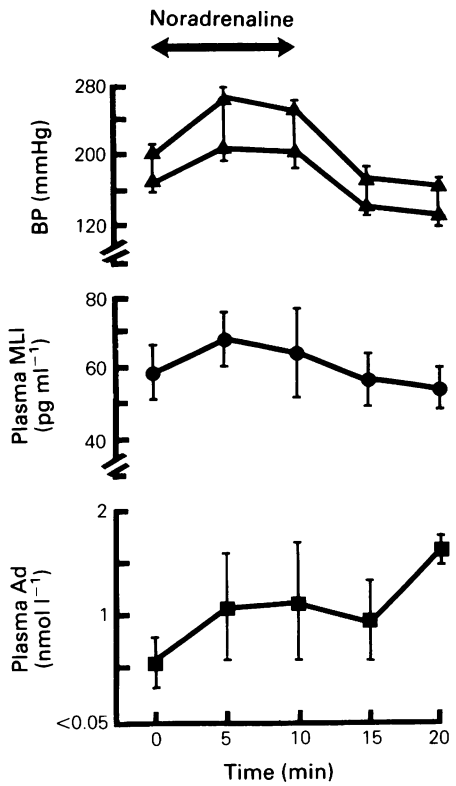

Figure 4 Blood pressure (BP) (▲), plasma [Met]enkephalin-like immunoreactivity (MLI) (●) and adrenaline (Ad) (■) responses to noradrenaline infusion (8 $\mu g\,kg^{-1}\,min^{-1}$) (←→) in anaesthetized greyhounds; mean values are shown with s.e.mean indicated by vertical bars, ($n = 3$).

include autonomic ganglia and sympathetic nerves (Di Giulio *et al.*, 1978) and gut (Polak *et al.*, 1977).

Little is known about the physiological, pathophysiological and/or pharmacological mechanisms that are associated with the release of MLI into the circulation. However, recently we have shown that circulating MLI levels rise following oral ethanol in chlorpropamide pretreated human subjects (Medbak *et al.*, 1981) and dogs (Medbak *et al.*, 1983). Moreover plasma MLI levels rise in greyhounds during insulin-induced hypoglycaemia (Medbak *et al.*, 1985) and artificial hyperventilation (Mason & Medbak, 1986).

To investigate whether neural mechanisms are important in the control and release of MLI, we compared the responses to nitroprusside followed, after a rest period, by a high dose of the ganglion blocker, hexamethonium. In those animals nitroprusside led to the expected fall in blood pressure together with elevation of plasma MLI and catecholamines. However, although hexamethonium produced significant hypotension it was not associated with any changes in plasma MLI (Figure 3). Moreover there was a fall in plasma noradrenaline and a rise in plasma adrenaline. These findings suggest that neural mechanisms are required, at least in part, for the release of MLI into the circulation during hypotension, and this was prevented by ganglion blockade by hexamethonium. The fall in plasma noradrenaline was probably due to the fact that the major part of circulating noradrenaline originates from the sympathetic nervous system whose activity would be inhibited by ganglion blockade. These results are in agreement with the report of Schadt & Gaddis (1985) who suggested that endogenous opioid peptides contained in the sympathetic nervous system share similar release mechanisms with noradrenaline. The rise in plasma adrenaline following hexamethonium was very much smaller than after nitroprusside although it was significant. The reason for this rise is not clear at present.

The next question we attempted to answer was the effect of hypertension on the circulating levels of MLI and catecholamines. We induced hypertension using two different drugs. The first was an angiotensin II infusion which produced a significant elevation in blood pressure (Figure 3). However, there was very little change in plasma MLI and noradrenaline whilst the plasma adrenaline levels rose significantly (Figure 3). As most (over 90%) of the catecholamine secreted by the adrenal medulla is adrenaline (Schadt & Gaddis, 1985) and most of the plasma noradrenaline originates from the sympathetic nervous system, it appears that rises in plasma adrenaline without associated changes in plasma noradrenaline are due to enhanced adrenomedullary activity. Angiotensin II is known to stimulate the adrenal medulla (Douglas, 1980) which explains our findings of elevated plasma adrenaline but not noradrenaline. However plasma MLI did not change, suggesting that the release of MLI from the adrenal medulla is less sensitive to angiotensin II. This is the second piece of evidence which suggests that the release of adrenaline and MLI from the adrenal medulla may be independent.

The second hypertensive agent we used was noradrenaline. Again it produced a significant elevation of blood pressure but it did not provoke any alterations in plasma MLI or adrenaline except at 20 min (for the latter) which is probably a response to the rebound hypotension that occurred 5–10 min after cessation of the noradrenaline infusion (Figure 4). These findings suggest that [Met]enkephalin is not involved in the tonic control of blood pressure.

[Met]enkephalin, like many peptides and hormones exists in the circulation in different molecular forms (Medbak *et al.*, 1983). In this study the molecular forms of circulating MLI have been characterized by

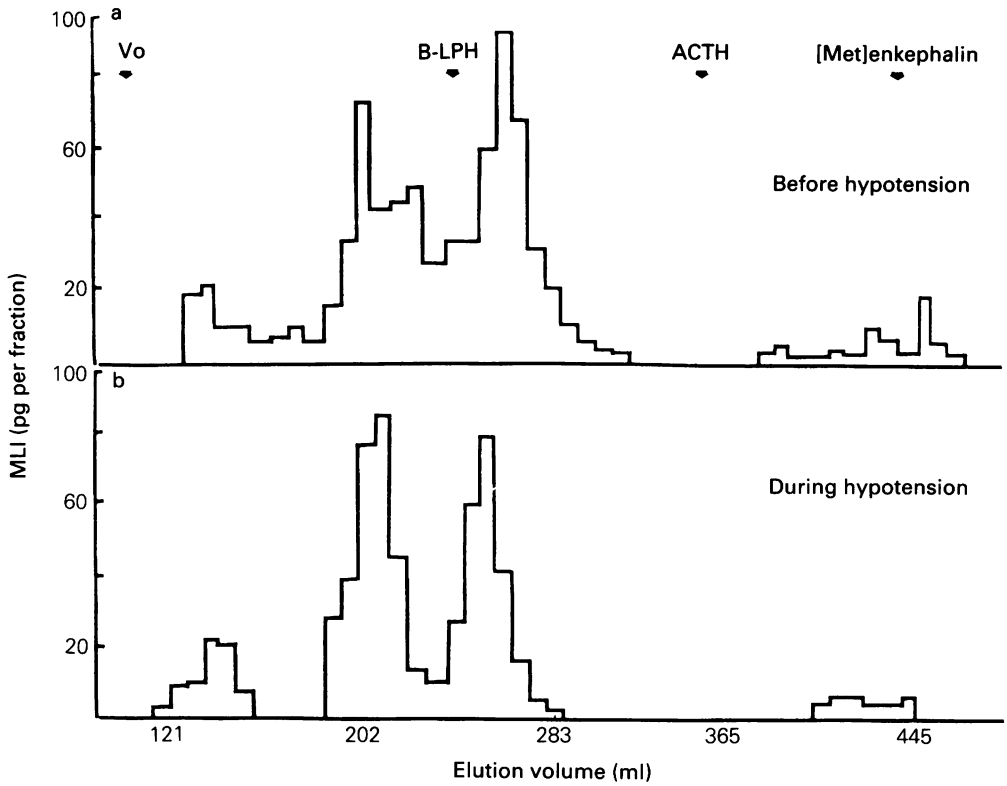

Figure 5 Sephadex G75 chromatography of plasma obtained before (a) and during (b) hypotension. ▼ denote elution positions of calibrating peptides. Vo, void volume; B-LPH, B-lipotrophic hormone; ACTH, adrenocorticotrophic hormone; [Met]enkephalin, methionine enkephalin.

gel filtration chromatography. This revealed that in both the basal and stimulated states the larger molecular forms of MLI with approximate molecular weights of 18 kD and 8 kD were the predominant forms compared to the smaller molecular forms (Figure 5). These forms are presumably derived from a large precursor molecule, proenkephalin, whose sequence has been predicted from the nucleotide sequence of its cDNA (Noda *et al.*, 1982). However, it is still not clear what is the function of these large molecular weight enkephalin-containing peptides.

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References

ATWEH, S.F. & KUMAR, M.J. (1977). Autoradiographic localisation of opiate receptors in rat brain. I. Spinal cord and lower medulla. *Brain Res.*, **124**, 53–67.

BOULOUX, P., PERRETT, D. & BESSER, G.M. (1985). Methodological considerations in the determination of plasma catecholamines by high-performance liquid chromatography with electrochemical detection. *Ann. Clin. Biochem.*, **22**, 194–203.

BURNIE, J. (1981). Naloxone in shock. *Lancet*, **i**, 942.

CLEMENT-JONES, V., LOWRY, P.J., REES, L.H. & BESSER, G.M. (1980). Development of a specific extracted radioimmunoassay for methionine enkephalin in human plasma and cerebrospinal fluid. *J. Endocrinol.*, **86**, 231–243.

COSTA, E., GUIDOTTI, A., HANBAUER, K. & SAIANI, L. (1983). Modulation of nicotinic receptor function by opiate recognition sites highly selective for met-enkephalin. *Fedn Proc.*, **42**, 2946–2952.

DI GIULIO, A.M., YANG, H.-Y.T., LUTOLD, B., FRATTA, M.,

HONG, J. & COSTA, E. (1978). Characterisation of enkephalin-like material extracted from sympathetic ganglia. *Neuropharmacology*, **17**, 989 992.

DOUGLAS, W.W. (1980). Polypeptides – angiotensin, plasma kinins, and others. In *The Pharmacological Basis of Therapeutics*. ed. Gilman, A.G., Goodman, L.S. & Gilman, A. pp. 647–667. New York: Macmillan.

EULIE, P.J. & RHEE, H.M. (1984). Reduction by phentolamine of the hypotensive effect of methionine enkephalin in anaesthetized rabbits. *Br. J. Pharmac.*, **83**, 783–790.

EVANS, S.F., MEDBAK, S., HINDS, C.J., TOMLIN, S.J., VARLEY, J.G. & REES, L.H. (1984). Plasma levels and biochemical characterisation of circulating met-enkephalin in canine endotoxin shock. *Life Sci.*, **34**, 1481–1486.

FENNESSY, M.R., & RATTRAY, J.F. (1971). Cardiovascular effects of intravenous morphine in the anaesthetized rat. *Eur. J. Pharmac.*, **14**, 1–8.

FLOREZ, J. & MEDIAVILLA, A. (1977). Respiratory and cardiovascular effects of met-enkephalin applied to the ventral surface of the brain stem. *Brain Res.*, **138**, 585–590.

GADDIS, R.R. & DIXON, W.R. (1982). Presynaptic opiate receptor mediated inhibition of endogenous nor-epinephrine and dopamine-β-hydroxylase release in cat spleen, independent of the presynaptic alpha-adrenoceptors. *J. Pharmac. exp. Ther.*, **223**, 77–83.

HANBAUER, I., GOVONI, S., MAJONE, E.A., YANG, H.-Y.T. & COSTA, E. (1982). In vivo regulation of the release of met-enkephalin-like peptides from dog adrenal medulla. In *Regulatory Peptides: From Molecular Biology to Function*. ed. Costa, E. & Trabucchi, M. pp. 209–215. New York: Raven Press.

HOLADAY, J.W. & FADEN, A.I. (1978). Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock. *Nature*, **275**, 450–451.

HOLADAY, J.W. (1983). Cardiovascular consequences of endogenous opiate antagonism. *Biochem. Pharmac.*, **32**, 573–585.

HUGHES, G.S.Jr. (1984). Naloxone and methylprednisone sodium succinate enhance sympathomedullary discharge in patients with septic shock. *Life Sci.*, **35**, 2319–2326.

KILPATRICK, D.L., JONES, B.N., LEWIS, R.V., STERN, A.S., KOJIMA, K., SHIVELY, J.E. & UDENFRIEND, S. (1982). An 18,200-dalton adrenal protein that contains four [met] enkephalin sequences. *Proc. natn. Acad. Sci. U.S.A.*, **77**, 1681–1685.

KUMAKURA, K., KAROUM, F., GUIDOTTI, A. & COSTA, E. (1980). Modulation of nicotinic receptors by opiate receptor agonists in cultured adrenal chromaffin cells. *Nature.*, **283**, 489–492.

LANG, R.E., BRUCKNER, U.B., HERMANN, K., KEMPF, B., RASCHER, W., STURM, V., UNGER, Th. & GANTEN, D. (1982). Effect of haemorrhagic shock on the concomitant release of endorphin and enkephalin-like peptides from the pituitary and adrenal gland in the dog. *Adv. Biochem. Psychopharmac.* ed. Costa, E. & Trabucchi, M. Vol. 33, pp. 363–369. New York: Raven Press.

LAUBIE, M., SCHMITT, H., VINCENT, M. & REMOND, G. (1977). Central cardiovascular effects of morphinomimetic peptides in dogs. *Eur. J. Pharmac.*, **46**, 67–71.

LAURENT, S., MARSH, J.D. & SMITH, T.W. (1985). Enkephalins have a direct positive inotropic effect on cultured cardiac myocytes. *Proc. natn. Acad. Sci. U.S.A.*, **82**, 5930–5934.

LEMAIRE, I., TSENG, R. & LEMAIRE, S. (1978). Systemic administration of β-endorphin: potent hypotensive effect involving a serotonergic pathway. *Proc. natn. Acad. Sci., U.S.A.*, **75**, 6240–6242.

MASON, D.F.J. & MEDBAK, S. (1986). Plasma met-enkephalin and catecholamines responses to artificial hyperventilation in anaesthetized greyhounds. *Br. J. Pharmac.*, Proc. Suppl., **88**, 315P.

MEDBAK, S., WASS, J.A.H., CLEMENT-JONES, V., COOKE, E.D., BOWCOCK, S.A., CUDWORTH, A.G. & REES, L.H. (1981). Chlorpropamide alcohol flush and circulating met-enkephalin: a positive link. *Br. med. J.*, **283**, 939–941.

MEDBAK, S., MASON, D.F.J. & REES, L.H. (1983). Chlorpropamide-ethanol induced met-enkephalin secretion in dogs: release mechanisms and biochemical characterization. *Regul. Peptides*, **7**, 195–206.

MEDBAK, S., MASON, D.F.J. & REES, L.H. (1985). Plasma met-enkephalin responses to insulin-induced hypoglycaemia in dogs. *J. Endocrinol.*, **107**, suppl., Abstract No. 65.

MOORE, R.H. & DOWLING, D.A. (1980). Effects of intravenously administered leu or metenkephalin on arterial blood pressure. *Regul. Peptides*, **1**, 77–87.

NODA, M., FURUTANI, Y., TAKAHASHI, H., TOYOSATO, M., HIROSE, T., INAYAMA, S., NAKANISHI, S. & NUMA, S. (1982). Cloning and sequence analysis of cDNA for bovine adrenal preproenkephalin. *Nature*, **295**, 202–206.

PETERS, W.R., JOHNSON, M.W., FRIEDMAN, P.A. & MITCH, W.E. (1981). Pressor effect of naloxone in septic shock. *Lancet*, **i**, 529–532.

POLAK, J.M., SULLIVAN, S.N., BLOOM, S.R., FACER, P. & PEARSE, A.G.E. (1977). Enkephalin-like immunoreactivity in the human gastrointestinal tract. *Lancet*, **i**, 972–974.

RUBIN, P.C. (1984). Opioid peptides in blood pressure regulation in man. *Clin. Sci.*, **66**, 625–630.

SANDER, G.E., GILES, T.D., KASTIN, A.J., QUIROZ, A.C., KANEISH, A. & COY, D.H. (1981). Cardiopulmonary pharmacology of enkephalins in the conscious dog. *Peptides*, **2**, 403–407.

SANDER, G.E. & GILES, T.D. (1982). Enkephalin analogs and dermorphin in the conscious dog: Structure-activity relationships. *Peptides*, **3**, 1017–1021.

SAPRU, H.N., WILLETTE, R.N. & KRIEGER, A.J. (1981). Stimulation of pulmonary 'J' receptors by an enkephalin-analog. *J. Pharmac. exp. Ther.*, **217**, 228–234.

SAUNDERS, W.S. & THORNHILL, J.A. (1985). No inotropic action of enkephalins or enkephalin derivatives on electrically-stimulated atria isolated from lean and obese rats. *Br. J. Pharmac.*, **85**, 513–522.

SCHADT, J.C. & GADDIS, R.R. (1985). Endogenous opiate peptides may limit norepinephrine release during haemorrhage. *J. Pharmac. exp. Ther.*, **232**, 656–660.

SCHAZ, K., STOCK, G., SIMON, W., SCHLOR, K-H., UNGER, T., ROCKHOLD, R. & GANTEN, D. (1980). Enkephalin effects on blood pressure, heart rate, and baroreceptor reflex. *Hypertension*, **2**, 395–407.

VIVEROS, O.H., DILIBERTO, E.J., HAZUM, E. & CHANG, K.-J. (1979). Opiate-like materials in the adrenal medulla. Evidence for storage and secretion with catecholamines.

Molec. Pharmac., **16,** 1101–1108.

WAMSLEY, J.K., ZARBIN, M.A., YOUNG, W.S. & KUHAR, M.J. (1982). Distribution of opiate receptors in the monkey brain: an autoradiographic study. *Neurosci.*, **7,** 595–613.

WEI, E.T., LEE, A. & CHANG, J.K. (1980). Cardiovascular effects of peptides related to the enkephalins and β-casomorphin. *Life Sci.*, **26,** 1517–1522.

YUKIMURA, T., STOCK, G., STUMP, H., UNGER, T. & GANTEN, D. (1981). Effects of (D-Ala^2)-methionine-enkephalin on blood pressure, heart rate and baroreceptor reflex sensitivity in conscious cats. *Hypertension*, **3,** 528–533.

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