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Reduction by phentolamine of the hypotensive effect of methionine enkephalin in anaesthetized rabbits

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1 In intact rabbits anaesthetized with pentobarbitone, methionine enkephalin (Met enkephalin, 1–1,000 $\mu g\,kg^{-1}$ i.v.) produced a dose-dependent bradycardia and hypotension.

2 The bradycardia and hypotension were antagonized by naloxone hydrochloride (1 $mg\,kg^{-1}$), but not by naloxone methobromide (1.3 $mg\,kg^{-1}$).

3 Phentolamine (1 and 4 $mg\,kg^{-1}$ i.v.) blocked both the hypotension and bradycardia produced by Met enkephalin.

4 The inhibitory effect of phentolamine was not due to a simple hypotensive action of this drug *per se* because a similar degree of hypotension induced by nitroprusside (15 $\mu g\,kg^{-1}$, i.v.) caused a further reduction of pressure when Met enkephalin was added.

5 Atropine (2 $mg\,kg^{-1}$) reduced the bradycardia but not the hypotensive response to Met enkephalin.

6 Met enkephalin did not antagonize the vasopressor effect of exogenous noradrenaline (2–8 $\mu g\,kg^{-1}$, i.v.).

7 Met enkephalin had no significant effects in superfused thoracic aortic strips and in isolated perfused hearts of rabbits.

8 It is concluded that the cardiovascular effects of Met enkephalin are more probably due to an action on the central nervous system, although a peripheral site of action cannot be completely excluded.

Introduction

The effects of methione enkephalin (Met enkephalin) on the cardiovascular system, especially on blood pressure, depend on the state of anaesthesia and route of drug administration. In general, Met enkephalin increases blood pressure in conscious rats (Schaz *et al.*, 1980), in conscious dogs (Sander & Giles, 1982) and in conscious cats (Yukimura *et al.*, 1981). However, in anaesthetized animals Met enkephalin either decreases or increases blood pressure depending upon the routes of drug administration. An intravenous injection of the drug decreased blood pressure in rats (Wei *et al.*, 1980), in dogs (Sander *et al.*, 1982) and in cats (Feldberg & Wei, 1977). An intracerebroventricular injection of the peptide into the lateral ventricle or the nucleus of tractus solatorius produced hypertension and tachycardia (Feldberg & Wei, 1977; 1978a,b; Petty & De Jong, 1983).

Most of the effects of Met enkephalin are antagonized by naloxone, suggesting an involvement of an opiate receptor in the cardiovascular effects of the peptide (Laubie *et al.*, 1977; 1979; Simon *et al.*, 1978; Sapru *et al.*, 1981). Although Farsang *et al.* (1980) suggested an involvement of central adrenoceptors in the cardiovascular effect of Met enkephalin in spontaneously hypertensive rats, the exact mechanism of the hypotensive action of the peptide is yet to be established. Therefore, the primary objective of the present study was to examine the effect of phentolamine on the hypotensive action of Met enkephalin in rabbits.

Part of this work was presented to the Federation of American Societies for Experimental Biology (Eulie & Rhee, 1983).

Methods

Male New Zealand white rabbits (Wischard Rabbitry, Sand Springs, Oklahoma; 1.5–3.5 kg) were

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anaesthetized with pentobarbitone (30 mg kg^{-1}), i.v.). For measurement of arterial pressure and delivery of drug the carotid (or femoral) artery, and jugular vein were cannulated, respectively. The animals were allowed to respire spontaneously and were maintained at 37°C with a temperature controlled body mat. Arterial pressure was monitored by a Statham P23 pressure transducer connected to a pressure processor amplifier (Gould Inst., Inc., Cleveland, Ohio) which electronically computes the heart rate, systolic, diastolic, mean, and pulse pressure. Central venous pressure and ECG (Standard Lead II) were recorded continuously on an electrostatic recorder (Gould Inst., model ES-1000).

The solutions of Met enkephalin were made in physiological saline (0.5 ml) just before the experiment. Various doses of Met enkephalin were injected via the jugluar vein, followed by 0.3 ml carrier. A dose-response was obtained with Met enkephalin in control animals and in animals pretreated (10 min before) with naloxone (1 mg kg^{-1}), naloxone methobromide (1.3 mg kg^{-1}), phentolamine (1 mg kg^{-1} or 4 mg kg^{-1}), atropine (2 mg kg^{-1}). Nitroprusside (15 μg kg^{-1}) or noradrenaline (2 to 8 μg kg^{-1}) were injected simultaneously with Met enkephalin.

In order to investigate the direct action of Met enkephalin on the heart, a retrograde perfusion of a rabbit isolated heart was used as previously described from this laboratory (Huang *et al.*, 1979). For the evaluation of the inotropic effect of Met enkephalin, the hearts were paced after removal of the atria. The hearts were perfused with Krebs-Henseleit solution (Winegrad & Shanes, 1962) alone or with increasing concentrations of Met enkephalin from 10^{-8} to 10^{-4} M. The effect of each concentration of Met enkephalin was evaluated for 10 min. We also injected the drug in decreasing concentration to minimize any potential development of tachyphylaxis to the peptide in some experiments.

Strips of the helically cut, descending aorta were prepared by the method of Furchgott (1960). Twelve strips were tested with noradrenaline (NA) alone and 6 strips were incubated with Met enkephalin 10^{-4} M for 10 min before being subjected to cumulatively increasing concentrations of NA. Only one dose-response was performed on each strip and 18 strips were assayed, 2 strips per animal.

The data in this study are expressed as the mean ± the standard error (± s.e.). Significant difference was considered as $P < 0.05$. The data from the *in vivo* studies were analyzed by two-way analysis of variance, and Students *t* test for paired data. A linear regression analysis using the method of least squares was performed on the data from the aortic strips only on the points that lay between 20 and 80% contraction to produce a best fit. The EC_{50} and 95% confidence intervals of the two curves were compared for overlap.

The drugs used in this study were: pentobarbitone sodium (W.A. Butler, Columbus, Ohio); Met enkephalin (Bioproducts, Belgium); naloxone HCl (Endo Laboratories, Garden City, New Jersey); phentolamine HCl (Ciba-Geigy, Summit, New Jersey); nitroprusside and atropine, free base (Sigma, St. Louis, Missouri); and naloxone methobromide (Boehringer Ingelheim, Ridgefield, Connecticut.

Results

In helical strips of rabbit aorta the EC_{50} for contractions produced by noradrenaline alone was 1.0×10^{-7} M. In this preparation, Met enkephalin at a concentration of 10^{-4} M had no significant effect on the shape of the dose-response curve to noradrenaline (data not shown). In the presence of Met enkephalin (10^{-4} M), the EC_{50} for noradrenaline was 1.3×10^{-7} M, which is statistically not different from

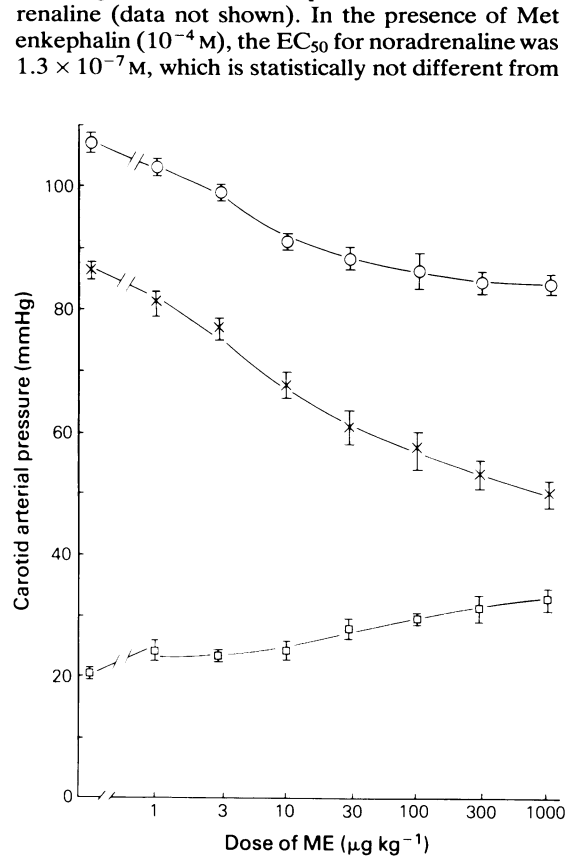

Figure 1 Effect of Met enkephalin (ME) on carotid arterial systolic (○), diastolic (×) and pulse pressure (□) in intact rabbits. Indicated doses of Met enkephalin were injected and the maximum effects on different blood pressures were tabulated. Each value represents mean of at least 5 animals; s.e.mean shown by vertical lines.

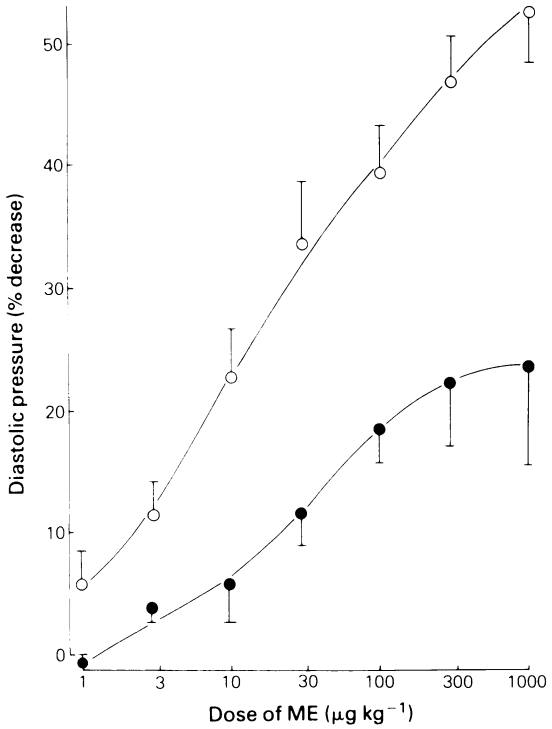

Figure 2 Inhibitory effect of naloxone on the hypotension induced by Met enkephalin(ME) in intact rabbits. Percentage decrease in diastolic blood pressure was calculated after different doses of Met enkephalin (○). This experiment was repeated in animals pretreated with naloxone (1 mg kg^{-1}) for 10 min (●). Each value represents mean of at least 5 experiments; s.e.mean shown by vertical lines.

the EC_{50} of noradrenaline without 10^{-4} M Met enkephalin. Met enkephalin at 10^{-4} M itself had no effect on the resting tension of the aortic strip.

In rabbit isolated perfused hearts, a wide range of Met enkephalin doses had no significant effect on heart rate. In paced and perfused hearts the left ventricular pressure and its first derivatives were not influenced by Met enkephalin at concentrations up to 10^{-4} M. Met enkephalin did not alter the contractile force of the right ventricle, measured directly by a strain guage arch sutured on the ventricle. Neither did Met enkephalin produce a significant change in the perfusion pressure (data not shown).

However, in intact animals Met enkephalin decreased blood pressure, especially the diastolic pressure. Decreases in systolic mean arterial pressure, and heart rate, and an increase in pulse pressure were also evident. The drug caused no abnormal change in the ECG and no significant change in the mean central venous pressure. The changes in blood pressure were dose-dependent (Figure 1). The peak response to Met enkephalin occurred between 15 and 20 s, independent of the dose; however, the duration exhibited dose-dependency. With 1,000 μg kg^{-1} of Met enkephalin the duration of the hypotension was 154 ± 4.9 s ($n = 5$) while the durations with 300, 100, 30, 10, 3, and 1 μg kg^{-1} of Met enkephalin were 122, 89, 81, 62, 40, and 33 s, respectively.

In order to assess involvement of the opiate receptor in the cardiodepressant action of Met enkephalin, animals were pretreated with naloxone, 1 mg kg^{-1}. Naloxone pretreatment, while having no effect on basal heart rate or arterial pressure, produced a significant ($P < 0.001$) antagonism of the decreases in heart rate and in diastolic pressure produced by Met enkephalin (Figures 2 and 3).

In an attempt to study the mechanism of the cardiovascular effect of Met enkephalin, a potential role of the autonomic nervous system was assessed with appropriate antagonists. Atropine alone (2 mg kg^{-1}) produced no significant effect on either heart rate or

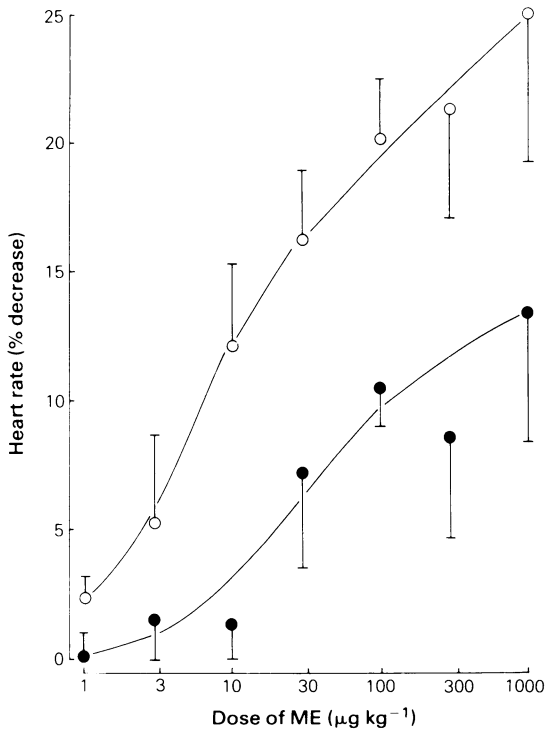

Figure 3 Naloxone blockade of the negative chronotropic effect of Met enkephalin (ME) in intact rabbits. Percentage decrease in heart rate in the animals was calculated in the group of Met enkephalin-treated (○) and in the group treated with Met enkephalin plus naloxone (●). Each value represents mean of at least 5 experiments; s.e.mean shown by vertical lines.

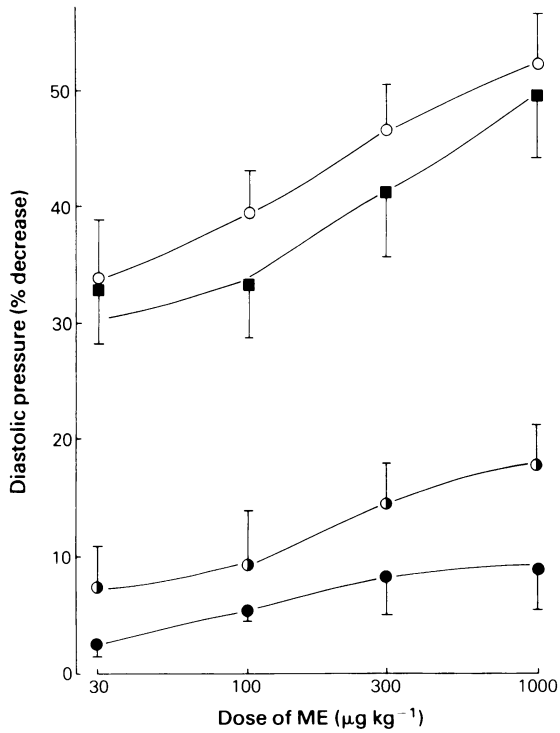

Figure 4 Effect of atropine and phentolamine on the hypotensive effect of Met enkephalin (ME) in intact rabbits. Hypotensive effect of Met enkephalin (○) was expressed as percentage reduction in carotid artery diastolic pressure. The effect of atropine (2 mg kg^{-1}) on the hypotensive action of Met enkephalin was tested (■). The effect of phentolamine, either 1 mg kg^{-1} (◑) or 4 mg kg^{-1} (●), on the hypotensive effect of Met enkephalin was evaluated 5 min after phentolamine treatments. Each value represents mean of at least 4 animals; s.e.mean shown by vertical lines.

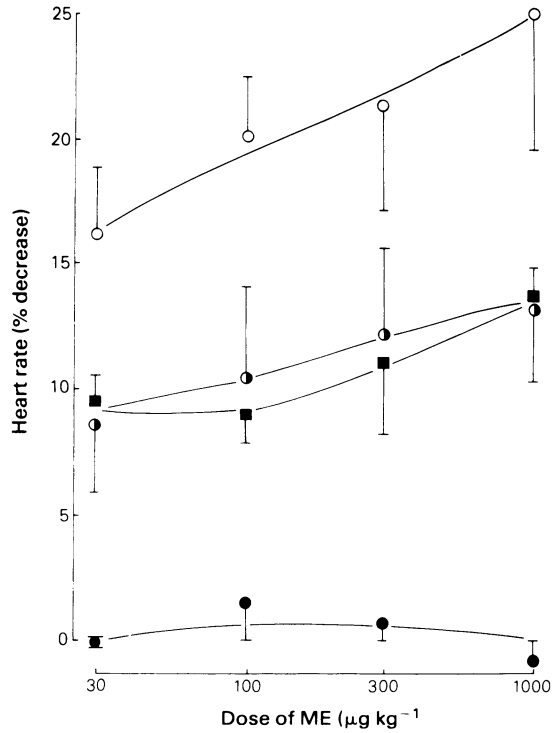

Figure 5 Effect of atropine (■) and phentolamine on the negative chronotropic effect of Met enkephalin (ME) in intact rabbits. Effect of atropine (2 mg kg^{-1}) and phentolamine, 1 mg kg^{-1}(◑) or 4 mg kg^{-1} (●) on percentage decrease in heart rate was evaluated. Met enkephalin was challenged 5 min after phentolamine treatment and each point represents mean of at least 4 animals; s.e.mean shown by vertical lines.

Table 1 Inability of naloxone methobromide to antagonize the hypotensive action of Met enkephalin in intact, anaesthetized rabbit

	% decrease in diastolic arterial blood pressure (%)[a]		
Dose of Met enkephalin (μg kg^{-1})	*Met enkephalin alone*	*Plus naloxone*[c] (1 mg kg^{-1})	*Plus naloxone methobromide* (1.3 mg kg^{-1})
1	9.4 ± 3.1[b]	4.2 ± 1.8	9.9 ± 6.1
10	18.2 ± 2.6	7.9 ± 3.7[d]	20.4 ± 3.6
100	31.4 ± 4.0	14.3 ± 7.1[d]	27.8 ± 8.0

[a] Changes in blood pressure were expressed as % of predrug value
[b] Each value represents mean ± s.e., of at least 5 animals.
[c] Both naloxone and naloxone methobromide were given 10 min before treatment with Met enkephalin as described under Methods.
[d] Indicates $P < 0.05$, compared to Met enkephalin-treated group.

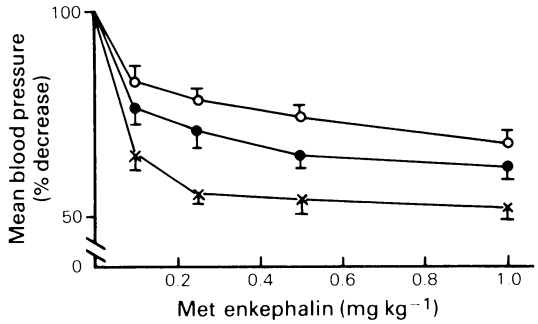

Figure 6 Additive effect of nitroprusside (15 μg kg^{-1}, i.v.) on the hypotensive action of Met enkephalin (ME) in intact rabbits. Hypotensive effect of Met enkephalin was evaluated as in Figure 1 with large doses of the peptide (●). Effect of phentolamine (4 mg kg^{-1}) on hypotensive action of the peptide was evaluated at 10 min after the treatment with phentolamine (○). Nitroprusside (×) was injected simultaneously with the peptide. Each value represents mean results from 6 rabbits, and both the effects of phentolamine and nitroprusside were significantly different from the effect of Met enkephalin alone; s.e.mean shown by vertical lines.

diastolic pressure in this species. Phentolamine alone at doses of 1 mg kg^{-1} produced no significant change in heart rate; however, it significant significantly ($P<0.025$) decreased diastolic pressure. A higher dose of phentolamine (4 mg kg^{-1}) decreased, though not significantly, heart rate (-46 ± 21 beats min^{-1}) and caused a significant ($P<0.025$) decrease in diastolic pressure (-14 ± 4 mmHg).

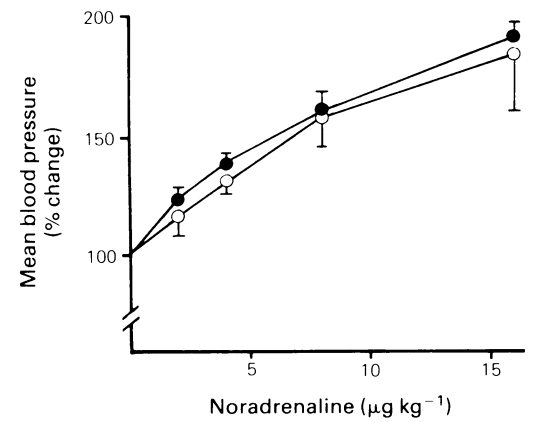

Figure 7 Inability of Met enkephalin to antagonize the hypertensive action of exogenous noradrenaline in intact rabbits. Effect of an intravenous injection of noradrenaline (2, 4, 8 and 16 μg kg^{-1}) was tested in a volume of 0.25 ml as in Figure 1 (●). Noradrenaline was also injected with Met enkephalin (500 μg kg^{-1},○) simultaneously in an identical manner. Each value represents mean of at least 5 rabbits and the paired points are not significantly different; s.e.mean shown by vertical lines.

Blood pressure after atropine, 2 mg kg^{-1}, was significantly ($P<0.02$) attenuated by Met enkephalin (Figure 4). Phentolamine, 4 mg kg^{-1}, blocked ($P<0.0001$) the decrease in diastolic pressure produced by Met enkephalin. A lesser dose, 1 mg kg^{-1}, also produced a highly significant ($P<0.001$) antagonism.

Figure 5 shows the effect of pretreatment with the autonomic blocking drugs on the decrease in heart rate induced by Met enkephalin. Atropine pretreatment (2 mg kg^{-1}) caused significant ($P<0.02$) attenuation of the bradycardia induced by Met enkephalin. Phentolamine (4 mg kg^{-1}) resulted in a highly significant ($P<0.0001$) blockade of the Met enkephalin-induced decrease in heart rate. A lower, 1 mg kg^{-1}, dose of phentolamine caused only a partial antagonism of Met enkephalin (Figure 5).

In order to test the central site of Met enkephalin action, naloxone methobromide, a quaternary morphine antagonist, was compared with naloxone in the inhibition of Met enkephalin's action. Naloxone methobromide did not block the hypotensive action of Met enkephalin (Table 1), although naloxone HCl blocked the cardiovascular actions of Met enkephalin at equimolar doses (Figures 2 and 3).

The inhibitory effect of phentolamine on the hypotensive action of Met enkephalin might be due to the consequential hypotensive action of phentolamine (4 mg kg^{-1}) since it reduced pressure by 15%. To test this possibility, nitroprusside (15 μg kg^{-1}), which reduced pressure to a similar extent to that observed with phentolamine, was injected simultaneously with Met enkephalin (Figure 6). Nitroprusside reduced pressure further with Met enkephalin instead of increasing pressure as in the case of phentolamine. Finally, Met enkephalin was tested against hypertension induced by exogenous noradrenaline. Met enkephalin (500 μg kg) was unable to reduce the hypertension produced by exogenous noradrenaline (Figure 7).

Discussion

Since the cardiovascular effects of Met enkephalin are complex and variable (see Introduction), the primary objective of this research was to examine the effect of the peptide on the peripheral cardiovascular system.

In helical strips of rabbit aorta, Met enkephalin itself caused no vascular relaxation, neither did it inhibit aortic contraction induced by noradrenaline. The peptide did not produce inotropic or chronotropic effects on the isolated perfused heart of the rabbit. The studies suggest that the peptide has minimal direct effect on the peripheral cardiovascular system of rabbits, although we have not tested the

effect of the peptide on any other resistance vessels. The results are in agreement with the observation on the effects of various narcotic drugs on contraction of papillary muscle (Rendig *et al.*, 1980). To our knowledge this is the only study on the effect of Met enkephalin in the rabbit perfused isolated heart.

In the intact rabbit, Met enkephalin caused a profound decrease in blood pressure that was not accompanied by a reflexogenic tachycardia but rather by a decrease in heart rate. Diastolic pressure decreased dramatically with a lesser, but significant, decrease in systolic pressure and a resultant increase in pulse pressure. This hypotensive action of the peptide was evident after suppression of sympathetic nerve discharge by the peptide (data not shown). These cardiovascular effects were dose-dependent and attenuated by naloxone, a specific opiate antagonist (Figures 2 and 3). Similar results have been found in the dog (Cowan *et al.*, 1976), the cat (McQueen & Ribeiro, 1980), and intact (Wei *et al.*, 1980) and decerebrate (Sapru *et al.*, 1981) rats.

Since the inhibitory effect of naloxone on the hypotensive effect of Met enkephalin may be mediated either centrally or peripherally, we studied the effect of naloxone metabromide, a quaternary derivative of naloxone, which does not readily cross the blood-brain barrier. At an equimolar dose, naloxone metabromide did not antagonize the cardiodepressant effects of the peptide, which suggests indirectly that the site of Met enkephalin action is in the central nervous system.

Recent studies suggest an involvement of opiate receptors in the regulation of blood pressure via central α-adrenoceptors (Schaz *et al.*, 1980; Laubie *et al.*, 1977; 1979; Farsang *et al.*, 1980). We therefore, tested the effect of Met enkephalin on blood pressure and heart rate after several autonomic blocking agents in intact rabbit hearts (Figure 4 and 5). Atropine ($2\,mg\,kg^{-1}$) produced a slight but significant antagonism of the hypotension and bradycardia produced by Met enkephalin. While Sapru *et al.* (1981) and Wei *et al.* (1980) also noted involvement of the parasympathetic system in the response in the rat, there seems to be a fundamental difference between the rabbit and the rat. In the rat, both groups noted that atropine blocked the decrease in heart rate. Our results in the rabbit show only a partial antagonism of the bradycardia.

The parasympathetic system probably plays a minimal role in the haemodynamic response to Met enkephalin in the rabbit. Phentolamine caused a total blockade of the hypotensive and bradycardiac effects of Met enkephalin (Figures 4 and 5). Clearly the sympathetic nervous system is integral in the cardiodepressant response to Met enkephalin. The results of this study, along with the inhibitory effect of the peptide on sympathetic renal nerve (Eulie, Peterson & Rhee, unpublished observations) and splanchnic nerve (Laubie *et al.*, 1977) discharge, strongly suggest that Met enkephalin is causing a marked withdrawal of sympathetic tone and, to a lesser degree, a possible concomitant increase in vagal activity. Since phentolamine ($4\,mg\,kg^{-1}$) alone decreased pressure as much as 15 to 20%, its inhibitory effect on the hypotensive action of Met enkephalin may be influenced by such a haemodynamic consequence. However, nitroprusside ($15\,\mu g\,kg^{-1}$ i.v.) at a dose that produces a comparable hypotension, intensified the hypotensive action of Met enkephalin (Figure 6). This provides evidence that the inhibitory effect of phentolamine is not due to mere haemodynamic consequence, but probably due to specific interaction between the two agents, even though the exact point of the interaction is not clear at this time.

In an effort to understand the site of Met enkephalin's interaction with noradrenaline, we finally tested the effect of the peptide on vasopressor responses to exogenous noradrenaline. The peptide obviously did not inhibit the vasopressor action of exogenous noradrenaline (Figure 7), although it was able to block the central discharge of adrenergic nerve firing (Eulie, Peterson & Rhee, unpublished observations).

While the results of this study fail to offer direct evidence that Met enkephalin acts centrally to modulate autonomic outflow, the evidence that we have does suggest that the dominant effect of Met enkephalin is on the autonomic nervous system. Since phentolamine blocked the decrease in heart rate in addition to the hypotension, a peripheral, post synaptic blockade by phentolamine provides an inadequate explanation for the dual antagonism. Therefore, a central antagonism by phentolamine is suggested and this view is reinforced by the effect of Met enkephalin on renal nerve activity and inability of naloxone methobromide to antagonize the effect (Table 1). Considering the documented presynaptic α_2 effect of phentolamine, it is not difficult to predict the antagonizing effect of phentolamine against the vasodepressor effect of Met enkephalin. Indeed, evidence has been presented that enkephalin reduces noradrenaline release from cerebrocortical adrenergic nerve endings (Taube *et al.*, 1976).

If Met enkephalin is acting centrally causing cardiodepression, then is there any evidence available that it crosses the blood-brain barrier? While enkephalins administered parenterally are known to have central effects (Plotnikoff *et al.*, 1976) and have been found to enter brain tissue readily in one study (Kastin *et al.*, 1976), Cornford *et al.*, (1978) and Rapoport and his colleagues (1980) have found that the opiate peptides have very low permeability into central sites. One possibility is that Met enkephalin enters discrete areas of the brain adjacent to

neurones involved in cardiovascular control such as the area postrema or the circumventricular organs that have fenestrated capillaries (Weindl & Joynt, 1972). Since subpopulations of opiate receptors have a high affinity for the enkephalins with receptors in the guinea-pig ileum (Lord *et al.*, 1977), rat and rabbit ileum, and bovine corpus striatum (Leslie *et al.*, 1980) having equilibrium dissociation constants in the order of 1 nM, then only trace amounts need interact with receptors in potential central sites.

This study provided clear evidence that the hypotensive action of Met enkephalin in rabbits is not due to a direct action either on the heart or on vascular muscle. Although the primary objective of the study was not focused on the exact site of Met enkephalin action, the results suggest a central site of action. However, at this time, we cannot rule out a peripheral site for Met enkephalin because morphine is known to decrease the release of noradrenaline from adrenergic terminals (Kayaalp & Kaymakcalan, 1966; Henderson & Hughes, 1974). Certain opiate agonists have been shown to have peripheral sympatholytic activities (Stickney & Eikenberg, 1981) and to modify the chronotropic action of noradrenaline in rat atria (Eiden & Ruth, 1982). Knoll (1976) also reported that rabbit median ear arteries exhibited relaxation subsequent to administration of leucine enkephalin. A more complete systematic investigation is necessary for the elucidation of the mechanism and site of action of Met enkephalin.

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