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No inotropic action of enkephalins or enkephalin derivatives on electrically-stimulated atria isolated from lean and obese rats

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1 Inotropic actions of the endogenous enkephalins, leucine enkephalin ([Leu] enkephalin) and methionine enkephalin ([Met] enkephalin), and derivatives, [D-Ala^2-methionine] enkephalinamide (DAMEA) and [D Ala^2-leucine]enkephalinamide (DALEA) were tested, alone or in combination with noradrenaline (NA), (±)-isoprenaline or carbachol, on electrically-stimulated atria excised from Sprague-Dawley, fatty, Zucker (fa/fa) and lean, hooded heterozygous (Fa/fa) rats.

2 [Met] enkephalin, [Leu] enkephalin, DAMEA and DALEA (4×10^{-7}M to 4×10^{-4}M) caused no significant changes in atrial tension in any group compared to pre-injection control values or those following the infusion of Krebs-Henseleit control solution.

3 NA and isoprenaline (10^{-7} to 10^{-6}M) caused significant, dose-related increases in atrial tension in each of the three strains of rats tested with the Fa/fa group showing the greatest change and fastest rate of tension development. [Met] enkephalin, [Leu] enkephalin, DAMEA or DALEA (4×10^{-6}M) infused concurrently with NA or isoprenaline (10^{-6}M) evoked atrial tension changes within each group that were not different from those observed when NA or isoprenaline was administered alone.

4 Carbachol (10^{-9} and 10^{-8}M) caused a dose-related decrease (10% and 30–40%, respectively, from pre-injection control values) in atrial tension in auricles excised from all three groups. Again, infusion of [Met] enkephalin, [Leu] enkephalin, DAMEA or DALEA (4×10^{-6}M) together with carbachol (10^{-8}M) did not affect atrial tension changes of auricles isolated from any group compared to when carbachol was given alone.

5 The results indicate that the endogenous pentapeptides, ([Met] or [Leu] enkephalin), or derivatives (DAMEA and DALEA) do not affect atrial tension of electrically-stimulated auricles isolated from Sprague-Dawley, fa/fa or Fa/fa rats. In addition, these pentapeptides do not modify the positive inotropic actions of NA or isoprenaline or the negative inotropic effects of carbachol. It is suggested that *in vivo*, the enkephalins or enkephalin derivatives do not have a direct action on the heart to alter myocardial contractility.

Introduction

In conscious, unrestrained rats central and peripheral injections of enkephalins or enkephalin derivatives evoke potent blood pressure changes (for review see Holaday, 1983). In our laboratory we have recently found that intravenous (i.v.) methionine enkephalin ([Met] enkephalin) and leucine enkephalin ([Leu] enkephalin) evoke pressor responses in conscious lean and obese rats, without increasing heart rate (unpublished observations) whereas the enkephalin derivatives ([D-Ala^2-methionine] enkephalinamide; DAMEA, and [D-Ala^2-leucine] enkephalinamide; DALEA) produce a potent depressor response associated with a marked bradycardia. Other investigators (Simon *et al.*, 1978; Ganten *et al.*, 1981) have described increases in heart rate together with the observed rise in mean arterial pressure (MAP) in conscious rats to [Met] and [Leu] enkaphalin. Much speculation has arisen as to where peripherally-administered enkephalins act to cause a change in blood pressure.

In vivo, circulating plasma enkephalins, derived from the adrenal gland (Viveros *et al.*, 1980) may alter cardiovascular function by acting either at peripheral sites (myocardium, peripheral vasculature, pulmonary chemoreceptors) or directly on brain receptors near

circumventricular regions, areas known to have a weaker blood-brain barrier (Holaday, 1983). Willette *et al.* (1982) have claimed that the fall in blood pressure in anaesthetized rats on administration of DAMEA is due to a pulmonary chemoreflex which inhibits control vasomotor activity. Using conscious dogs Sander *et al.* (1981) proposed that enkephalins produce a vasoconstrictor effect. In rats, this vasoconstrictor effect may also hold true for intra-arterial as well as i.v.-administered [Met] and [Leu] enkephalin; both cause an increase in MAP with no associated tachycardia (unpublished observations). As mentioned, some *in vivo* studies (Simon *et al.*, 1978; Ganten *et al.*, 1981) postulate that the pressor responses to [Met] and [Leu] enkephalin are due to the associated tachycardia seen in the conscious rats, but *in vitro* studies (Eiden & Ruth, 1982; Ruth & Eiden, 1984) indicate that enkephalins have no effects on the heart rate of isolated, spontaneously beating rat atria. However, [Leu] and [Met] enkephalin were shown to attenuate the positive chronotropic effects of noradrenaline and isoproterenol.

From previous studies in which endogenous opioids were given to shocked animals (Faden *et al.*, 1980) or administered directly into the nucleus tractus solitarius of anaesthetized rats (Hassen *et al.*, 1982), it has been speculated that these pentapeptides may affect myocardial contractility to bring about the observed changes in systemic arterial pressure. To date, the direct effects of endogenous opioids on myocardial contractility have not been investigated. Thus, the purpose of the present study was to determine if enkephalins ([Met] and [Leu] enkephalin) or enkephalin analogues (DAMEA and DALEA), on their own, produce any changes in auricular contractility of electrically-stimulated atria isolated from Sprague-Dawley or fatty, Zucker rats or lean, hooded heterozygous littermates. In addition, we wished to investigate the effects of the pentapeptides on the positive inotropic effects of noradrenaline and isoprenaline or the negative inotropic actions of cholinomimetic agents like carbachol. Obese, Zucker rats, which have high plasma and pituitary levels of endogenous opioids (Margules *et al.*, 1978), have been shown to have a reduced inotropic response to secretin and (±)-isoprenaline, compared to lean controls (Chatelain *et al.*, 1981; Robberecht *et al.*, 1983).

Methods

Animals

Groups of male Sprague-Dawley (approx. 300 g); lean, hooded heterozygous (Fa/fa; approx. 300 g) and age-matched fatty, Zucker rats (fa/fa; approx. 450 g) were used in this study. The Sprague-Dawley rats were obtained from Charles River Labs (Montreal) and the Fa/fa and fa/fa rats were bred locally in our department from breeding stock originally purchased from Vassar College, Poughkeepsie, N.Y. These rats were kept in a controlled, environmental room (ambient temperature 21°C, relative humidity≈30%) which had a 12 h on-off lighting schedule. Animals were fed Purina Rat Chow and had free access to tap water.

In vitro *isolated auricle preparation*

Following a stunning blow to the head the heart of each rat was immediately excised and placed in oxygenated Krebs-Henseleit solution (bicarbonate buffer) of the following composition (mM) NaCl 118, KCl 5.1, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25, $MgSO_4$ 1.2, D-glucose 11.1. The right auricle was removed (diameter≈2 mm) and suspended under 8–9 g tension at 37°C in a 40 ml tissue bath containing the Krebs-Henseleit solution (pH 7.35) aerated with 95% O_2, 5% CO_2. Each auricle was stimulated electrically via SD9B Grass stimulator at a voltage at least 5 V above maximum (20 V absolute), with a pulse duration of 20 ms and with a 6.0 Hz frequency (360 contractions min^{-1} to mimic resting heart rate of unstressed rats). The preparation was allowed to equilibrate in these conditions for at least 30 min before drug testing. Measurements of atrial contractility were done with a force displacement transducer (Model FTO3C) connected to a 7B Grass rectilinear recorder.

After equilibration, Krebs-Henseleit solution, the β-adrenoceptor agonists, carbachol, enkephalins, enkephalin derivatives were injected randomly (1 ml total volume) into the tissue bath. Atrial contractility was monitored immediately before and for 90 s post-injection. At least 20 min elapsed between subsequent injections. The following drugs were tested alone for their effects on atrial contractility: noradrenaline HCl (NA; Sigma), (±)-isoprenaline HCl (Sigma), carbamylcholine chloride (Sigma), methionine enkephalin acetate ([Met] enkephalin; Sigma), leucine enkephalin acetate ([Leu] enkephalin; Sigma), [D-Ala^2-methionine] enkephalinamide acetate (DAMEA; Sigma), [D-Ala^2-leucine] enkephalinamide acetate (DALEA; Sigma). Then, the enkephalins and enkephalin derivatives were injected with the adrenoceptor or cholinoceptor agonists (NA, isoprenaline and carbachol) to determine if the endogenous opioids affected the positive or negative inotropic actions of these agents.

Analysis of data

Tension (mg) developed by the isolated auricle preparation was calibrated before and after the completion of the experiments using a set of known

stainless steel weights. Differences in atrial contractility were analysed using a one way analysis of variance. Statistically significant differences between data were considered to have a P value of <0.05.

Results

Table 1 shows the mean percentage changes in atrial tension, compared to pre-injection control values, of electrically-stimulated auricles isolated from Sprague-Dawley, fa/fa or Fa/fa rats following an infusion of Krebs-Henseleit solution or various concentrations (4×10^{-7}M to 4×10^{-4}M) of [Met] enkephalin, [Leu] enkephalin, DAMEA or DALEA. Infusion of 1 ml of Krebs-Henseleit solution to any of the three groups of rats resulted in no significant changes in atrial tension over 90 s post-administration, compared to pre-injection control tension levels, as tension only changed from +2 to −5% from control values. Interestingly, when 4×10^{-7}M to 4×10^{-5}M [Met] enkephalin, [Leu enkephalin, DAMEA or DALEA were given to the three groups of rats again no statistical increases or decreases in atrial tension were seen in any group compared to either the respective pre-injection control values within a particular group or the values during infusion of Krebs-Henseleit solution. When a concentration of 4×10^{-4}M of these pentapeptides were tested in some animals, no increases in atrial tension occurred but instead there was a tendency towards a reduction in atrial contractility, a trend more evident following an infusion of DAMEA or DALEA.

Figure 1 shows the mean % changes in atrial tension of electrically-stimulated auricles isolated from Sprague-Dawley rats over a 90 s period following infusion of noradrenaline (NA) or (±)-isoprenaline or when these agents were given together with the pentapeptides. Dose-related increases in atrial tension occurred following infusion of NA or isoprenaline. In this group of rats, 10^{-6}M NA caused a maximum change in atrial tension of 98 ± 13% 30s post-administration (or maximum absolute change of 115 ± 8 mg tension compared to pre-injection controls) and the same concentration of isoprenaline resulted in a 115 ± 18% increase in tension (or maximum absolute change of 135 ± 30 mg tension) 30 s post-administration. When each of the pentapeptides (4×10^{-6}M) were infused with either NA (10^{-6}M, Figure 1b) or isoprenaline (10^{-6}M, Figure 1d), no significant changes in atrial tension were observed compared to when either NA or isoprenaline were given alone. At 30 s

Table 1 Tension changes in atrial contractility of electrically-stimulated auricles isolated from groups ($n = 6$) of Sprague-Dawley, fatty, Zucker (fa/fa) and lean, heterozygous rats (Fa/fa) 30, 60 and 90 s after administration of enkephalins or enkephalin derivatives

Agent	*Time after administration* (s)								
	Sprague-Dawley			*Fa/fa*			*Fa/fa*		
	30	*60*	*90*	*30*	*60*	*90*	*30*	*60*	*90*
Krebs-Henseleit	−3 ± 3	2 ± 3	1 ± 1	−5 ± 3	1 ± 5	1 ± 4	−2 ± 3	−4 ± 3	−4 ± 5
Methionine enkephalin									
4×10^{-7}M[1]	−3 ± 4	1 ± 6	2 ± 5	−5 ± 6	−6 ± 1	3 ± 3	−3 ± 3	−2 ± 5	−2 ± 6
4×10^{-6}M	−3 ± 2	−3 ± 2	−3 ± 4	−12 ± 5	−8 ± 7	−7 ± 5	−4 ± 3	−5 ± 5	−2 ± 4
4×10^{-5}M	3 ± 3	0 ± 0	−2 ± 2	−1 ± 7	−5 ± 3	−5 ± 3	−4 ± 8	−5 ± 3	−6 ± 3
4×10^{-4}M ($n = 1$)				−10	0	−10	−7	0	−7
Leucine enkephalin									
4×10^{-7}M	0 ± 7	−2 ± 6	1 ± 5	−11 ± 9	−13 ± 9	−16 ± 8	2 ± 6	−2 ± 6	−7 ± 3
4×10^{-6}M	−1 ± 1	0 ± 3	−1 ± 5	0 ± 0	−1 ± 2	−3 ± 3	0 ± 0	2 ± 2	−2 ± 2
4×10^{-5}M	4 ± 9	6 ± 14	6 ± 14	−9 ± 7	−10 ± 6	−12 ± 6	−10 ± 6	−8 ± 6	−6 ± 6
4×10^{-4}M ($n = 1$)				0	0	0	−9	−9	−9
[D-Ala^2-Met] enkephalinamide									
4×10^{-7}M	0 ± 4	−2 ± 2	−5 ± 3	−7 ± 3	−11 ± 4	−10 ± 5	−4 ± 2	−0 ± 3	5 ± 5
4×10^{-6}M	6 ± 6	10 ± 5	6 ± 3	−8 ± 6	−8 ± 6	−8 ± 6	−6 ± 2	−2 ± 6	−4 ± 5
4×10^{-5}M	−4 ± 3	5 ± 4	4 ± 6	−7 ± 4	−4 ± 5	−6 ± 5	−2 ± 2	6 ± 2	1 ± 4
4×10^{-4}M ($n = 1$)							−16	−16	−16
[D-Ala^2-Leu] enkephalinamide									
4×10^{-7}M	−3 ± 4	8 ± 6	6 ± 0	1 ± 13	1 ± 13	−5 ± 6	3 ± 4	3 ± 7	0 ± 7
4×10^{-6}M	7 ± 3	2 ± 2	3 ± 5	−3 ± 3	7 ± 4	3 ± 4	−6 ± 6	−4 ± 3	−8 ± 3
4×10^{-5}M	7 ± 4	4 ± 4	7 ± 4	−9 ± 2	−8 ± 6	−6 ± 4	−8 ± 1	−5 ± 3	−6 ± 4
4×10^{-4}M ($n = 1$)							−12	−12	−12

Values shown are mean % change in atrial tension (± s.e.mean) from control levels.[1] Final bath concentration.

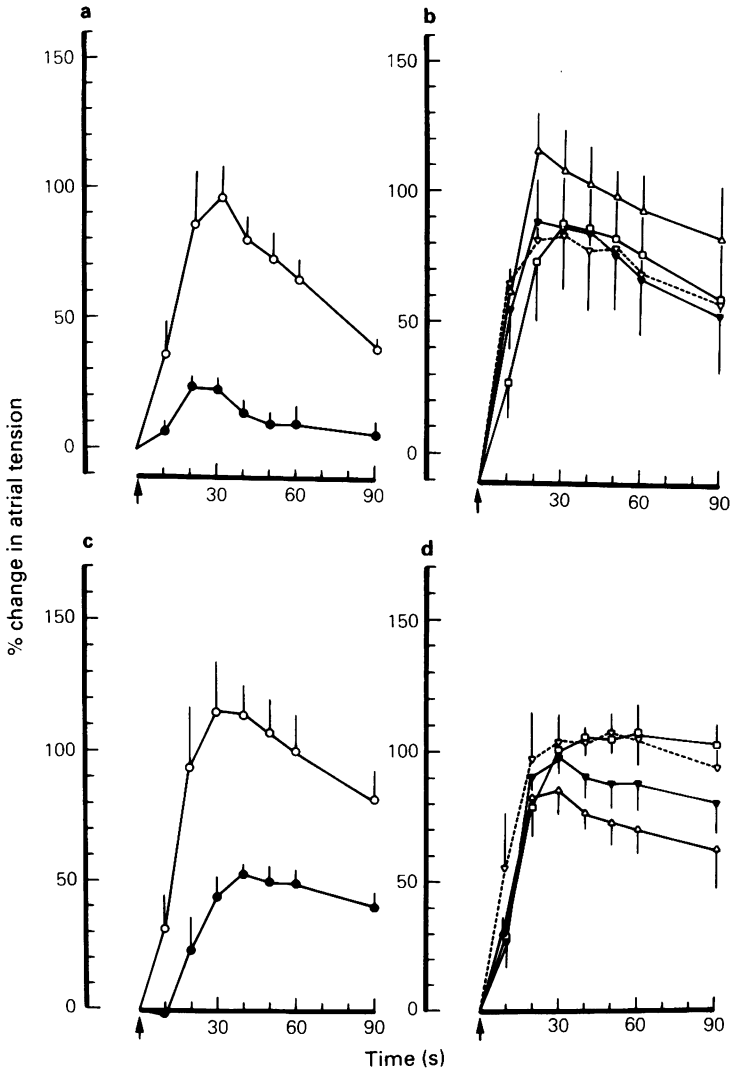

Figure 1 Mean % change in atrial tension from pre-injection control, of electrically-stimulated auricles isolated from Sprague-Dawley rats. Tension changes are shown after the infusion of a final bath concentration of: (a) noradrenaline (NA) 10^{-7}M (●), 10^{-6}M (○; (b) NA 10^{-6}M + [Met] enkephalin (ME) 4×10^{-6}M (▼), NA 10^{-6}M + [Leu] enkephalin (LE) 4×10^{-6}M (□), NA 10^{-6}M + [D-Ala^2-methionine] enkephalinamide (DAMEA), 4×10^{-6}M (△), NA 10^{-6}M + [D-Ala^2-leucine] enkephalinamide (DALEA) 4×10^{-6}M (▽); (c) isoprenaline (Iso) 10^{-7}M (●), 10^{-6}M (○); (d) Iso 10^{-6}M + ME 4×10^{-6}M (▼), Iso 10^{-6}M + LE 4×10^{-6}M (□), Iso 10^{-6}M + DAMEA 4×10^{-6}M (△), Iso 10^{-6}M + DALEA 4×10^{-6}M (▽). Vertical lines show s.e.mean; $n = 6$ rats.

post-infusion, atrial tension increased 86 ± 19% compared to pre-injection controls (mean absolute change of 90 ± 11 mg) when NA plus [Met] enkephalin were given, 88 ± 38% (80 ± 11 mg) with NA plus [Leu] enkephalin, 109 ± 14% (110 ± 9 mg) following NA plus DAMEA, and 83 ± 20% (93 ± 16 mg) when NA plus DALEA were given. When the pentapeptides were combined with isoprenaline, atrial tension rose 97 ± 5% from pre-injection controls (mean absolute tension change of 103 ± 10 mg) 30 s following an infusion of isoprenaline plus [Met] enkephalin, 104 ± 3% (107 ± 16 mg) with isoprenaline plus [Leu enkephalin LE, 106 ± 12% (120 ± 12 mg) with isoprenaline plus DAMEA and 86 ± 10% (97 ± 4 mg)

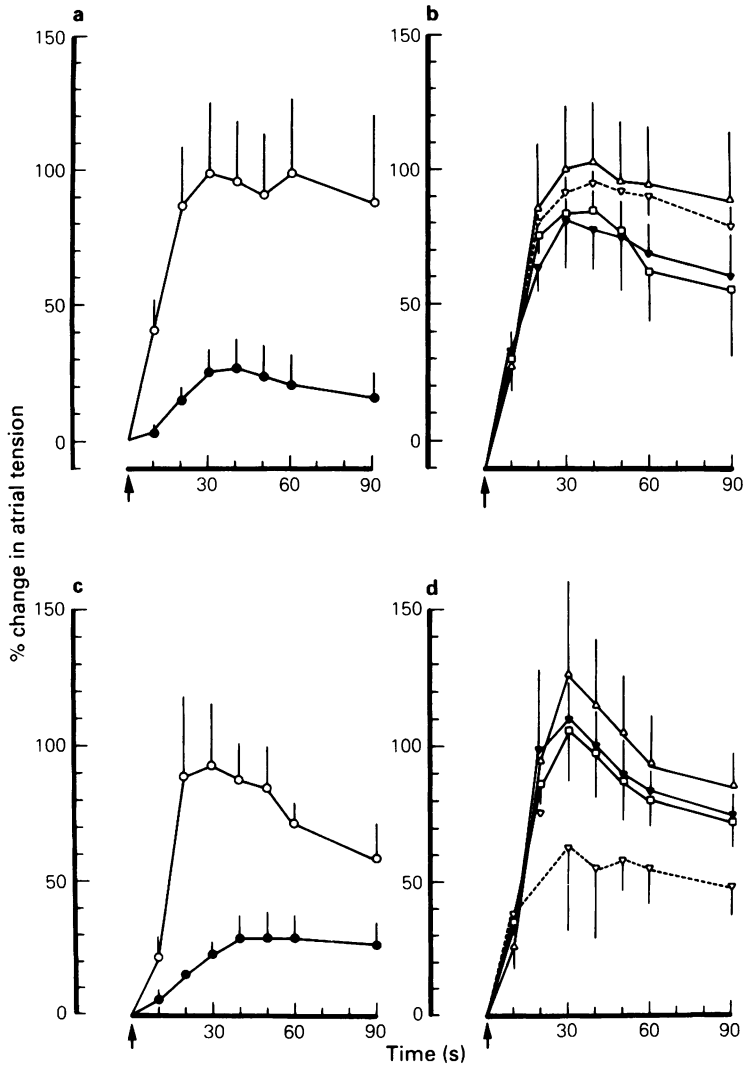

Figure 2 Mean % change in atrial tension from pre-injection control, of electrically-stimulated auricles isolated from fatty, Zucker (fa/fa) rats. Tension changes are shown after infusion of a final bath concentration of: (a) NA 10^{-7}M (●) 10^{-6}M (○); (b) NA 10^{-6}M + ME 4×10^{-6}M (▼), NA 10^{-6}M + LE 4×10^{-6}M (□), NA 10^{-6}M + DAMEA 4×10^{-6}M (△), NA 10^{-6}M + DALEA 4×10^{-6}M (▽); (c) Iso 10^{-7}M (●), 10^{-6}M (○); (d) Iso 10^{-6}M + ME 4×10^{-6}M (▼), Iso 10^{-6}M + ME 4×10^{-6}M (□), Iso 10^{-6}M + DAMEA 4×10^{-6}M (△), Iso 10^{-6}M + DALEA 4×10^{-6}M (▽). Abbreviations, as in legend to Figure 1. Vertical lines show s.e.mean; $n = 6$ rats.

with isoprenaline plus DALEA.

When NA or isoprenaline were superfused over electrically-stimulated auricles isolated from fatty, Zucker rats (Figure 2a and c) dose-related increases in atrial tension again occurred. NA (10^{-6}M) had increased atrial tension by 99 ± 27% from control (105 ± 10 mg) by 30 s post-administration in this group and 10^{-6}M isoprenaline increased atrial tension development by 93 ± 24% (169 ± 42 mg) by the same time after infusion. When the pentapeptides (4×10^{-6}M) were given in conjunction with NA (10^{-6}M) (Figure 1b) the tension changes evoked were not different from those observed when NA was administered alone. By 30 s post-infusion NA plus [Met] enkephalin caused atrial tension to increase by 81 ± 20%; with NA plus [Leu] enkephalin it in-

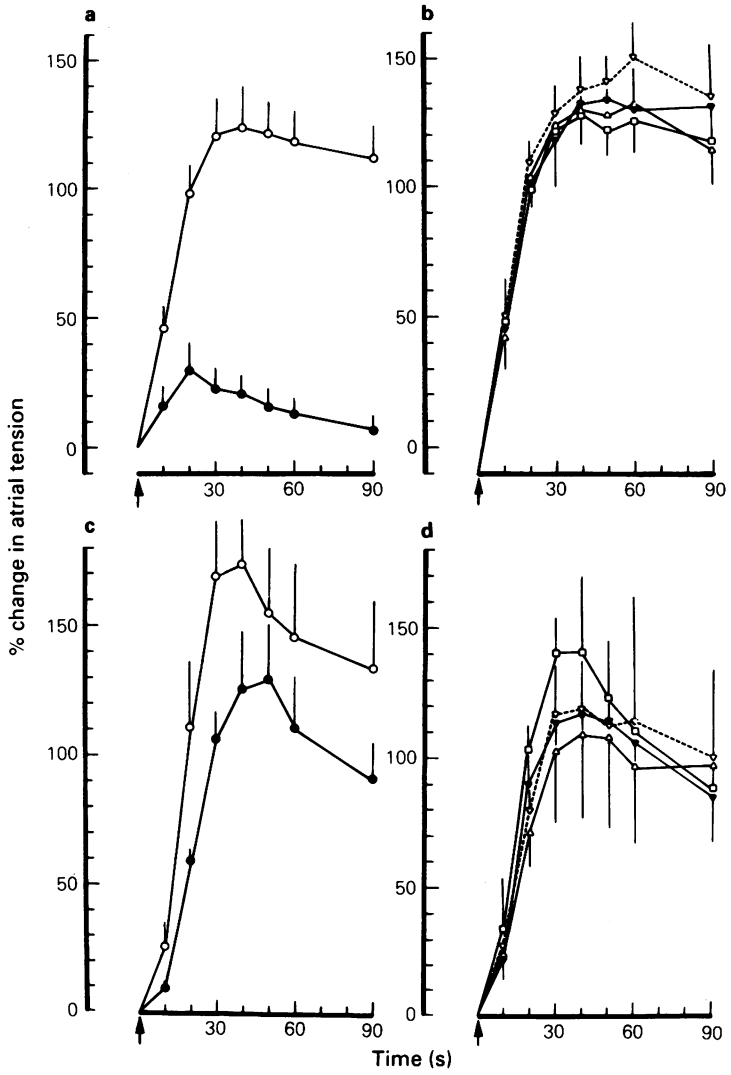

Figure 3 Mean % change in atrial tension from pre-injection control, of electrically-stimulated auricles isolated fro lean, heterozygous littermates of fatty, Zucker (fa/fa) rats. Tension changes are shown after infusion of a final ba concentration of: (a) NA 10^{-7}M (●), 10^{-6}M (○); (b) NA 10^{-6}M + ME 4×10^{-6}M (▼), NA 10^{-6}M + LE 4×10^{-6} (□), NA 10^{-6}M + DAMEA 4×10^{-6}M (△), NA 10^{-6}M + DALEA 4×10^{-6}M (▽); (c) Iso 10^{-7}M (●), 10^{-6}M (○); (Iso 10^{-6}M + ME 4×10^{-6}M (▼), Iso 10^{-6}M + ME 4×10^{-6}M (□), Iso 10^{-6}M + DAMEA 4×10^{-6}M (△), I 10^{-6}M + DALEA 4×10^{-6}M (▽). Abbreviations, as in legend to Figure 1. Vertical lines show s.e.mean; $n = 6$ rat

creased by 83 ± 22%; with NA plus DAMEA by 100 ± 43% and with NA plus DALEA by 92 ± 37%. Similarly, no significant changes in atrial tension were found when isoprenaline was infused with the pentapeptides (4×10^{-6}M). Atrial tension increased 110 + 35% from pre-injection controls at 30 s post-infusion when isoprenaline plus [Met] enkephalin were administered, 106 ± 26% with isoprenaline plus [Leu] enkephalin, 127 ± 42% with isoprenaline plus DAMEA and 63 ± 29% with isoprenaline DALEA (Figure 2d).

Atrial tension increased 120 ± 17% from pre tion controls (151 ± 18 mg) 30 s after the infus NA (10^{-6}M) over electrically-stimulated a isolated from the group of lean heterozygous ra fa) Figure 3a). With 10^{-7}M NA the maxim increase in atrial tension was 23 ± 9% (25 : from control values) at 30 s post-infusion. E

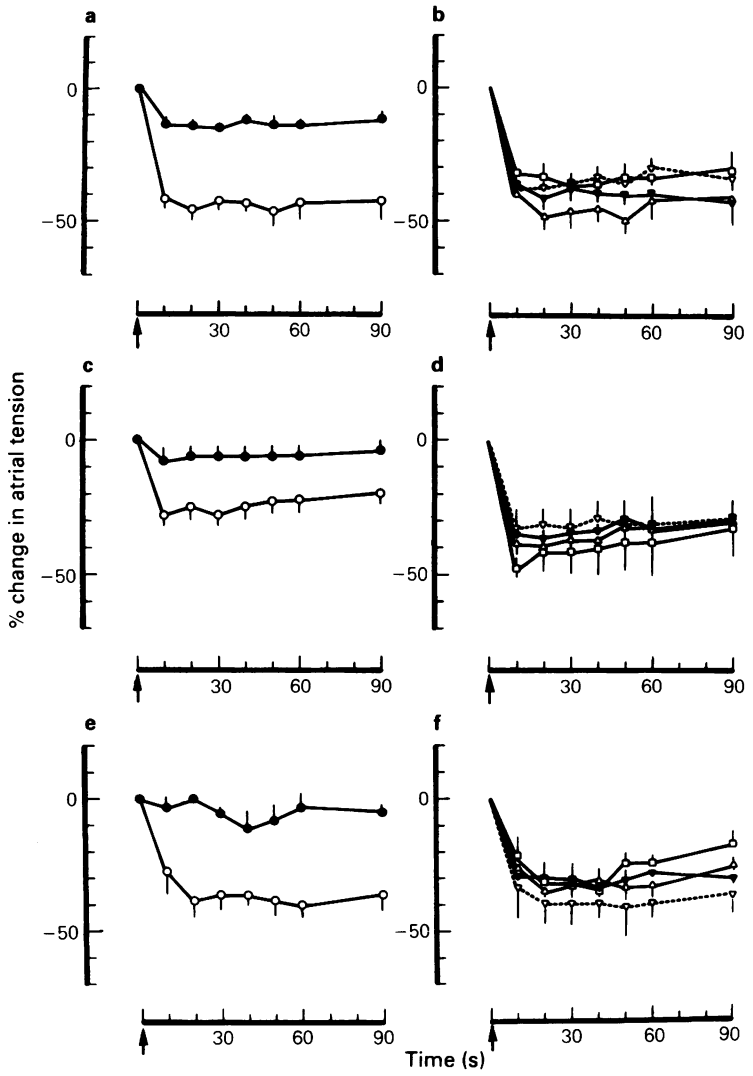

Figure 4 Mean % change in atrial tension from pre-injection control, of electrically-stimulated auricles isolated from groups ($n = 6$) of Sprague-Dawley (a and b), fatty, Zucker (c and d) and lean heterozygous littermates (e and f). Tension changes are shown after the infusion of a final bath concentration of; (a) Carbachol (Carb) 10^{-9}M (●), 10^{-8}M (○); (b) Carb 10^{-8}M + ME 4×10^{-6}M (▼), Carb 10^{-8}M + LE 4×10^{-6}M (□), Carb 10^{-8}M + DAMEA 4×10^{-6}M (△), Carb 10^{-8}M + DALEA 4×10^{-6}M (▽); (c) Carb 10^{-9}M (●), 10^{-8}M (○); (d) Carb 10^{-8}M + ME 4×10^{-6}M (▼), Carb 10^{-8}M + LE 4×10^{-6}M (□), Carb 10^{-8}M + DAMEA 4×10^{-6}M (△), Carb 10^{-8}M + DALEA 4×10^{-6}M (▽); (e) Carb 10^{-9}M (●), 10^{-8}M (○); (f) Carb 10^{-8}M + ME 4×10^{-6}M (▼), Carb 10^{-8}M + LE 4×10^{-6} (□), Carb 10^{-8}M + DAMEA 4×10^{-6}M (△), Carb 10^{-8}M + DALEA 4×10^{-6}M (▽). Abbreviations, as in legend to Figure 1. Vertical lines show s.e.mean.

post-administration isoprenaline (10^{-7} and 10^{-6}M) increased atrial tension 126 ± 22% (194 ± 12 mg) and 174 ± 41% (256 ± 30 mg) from respective controls (Figure 3c). When the pentapeptides (4×10^{-6}M) were combined with NA (10^{-6}M) in this Fa/fa group, atrial tension development was unchanged from that seen when 10^{-6}M NA was added alone. By 30 s post-administration atrial tension increased 118 ± 26% (156 ± 10 mg) with NA plus [Met] enkephalin; 123 ± 15% (164 ± 8 mg) with NA plus [Leu] enkephalin; 123 ± 12% (160 ± 8 mg) with NA plus DAMEA and 127 ± 22% (159 ± 11 mg) with NA plus

DALEA. When isoprenaline was given with each pentapeptide atrial tension tended to be reduced but no significant changes occurred compared to when isoprenaline (10^{-6}M) was administered alone (e.g. with isoprenaline plus [Met] enkephalin atrial tension had increased 116 ± 25% (153 ± 32 mg) by 40 s post-infusion, 140 ± 30% (194 ± 26 mg) with isoprenaline plus [Leu] enkephalin, 109 ± 32% (156 ± 57 mg) with isoprenaline plus DAMEA and 117 ± 56% (166 ± 82 mg) with isoprenaline plus DALEA).

Figure 4 shows the % changes in atrial tension, from pre-injection controls, of electrically-stimulated auricles isolated from groups of Sprague-Dawley, fa/fa and Fa/fa rats after carbachol or when carbachol plus one of the pentapeptides was added to the tissue bath. Dose-related decreases in atrial tension occurred in the Sprague-Dawley (a), fa/fa (c) and Fa/fa rats (e) with 10^{-9} and 10^{-8}M carbachol. With 10^{-9}M carbachol atrial tension decreased approximately 10% in all groups whereas a 30–40% reduction in atrial tension was observed with 10^{-8}M carbachol. With both concentrations of carbachol, atrial tension remained below pre-injection control levels and relatively unchanged over the whole 90 s recording period. As with NA and isoprenaline, combining the pentapeptides (4×10^{-6}M) with carbachol (10^{-8}M) did not significantly affect the changes in atrial tension occurring, in any of the isolated auricles from the three groups of rats tested, in response to carbachol (10^{-8}M).

Table 2 shows the mean rate change in atrial tension measured over a 30 s period following the infusion of either NA, isoprenaline or carbachol alone, or in combination with either of the 4 pentapeptides, in auricles isolated from Sprague-Dawley, fa/fa and Fa/fa rats. The rate of atrial tension development of auricles isolated from the Fa/fa group given either NA or isoprenaline (10^{-6}M) was significantly greater than that seen when these positive inotropic agents were given to either the Sprague-Dawley or fa/fa groups of rats. Within each of the 3 strains of rats tested, the addition of 4×10^{-6}M [Met] or [Leu] enkephalin, DAMEA or DALEA together with either NA, isoprenaline or carbachol did not significantly alter the rate of atrial tension change from the seen when either NA, isoprenaline or carbachol was given alone.

Discussion

In vitro studies have shown that gut peptides such as glucagon (Farah *et al.*, 1984) secretin (Chiba, 1976) and vasoactive intestinal peptide (Said *et al.*, 1972) have positive inotropic actions. The present study shows that the endogenous enkephalins ([Met] or [Leu] enkephalin) or derivatives (DAMEA and DALEA), peptides known to evoke potent blood pressure changes in conscious rats (Schaz *et al.*, 1980; Thornhill & Saunders, unpublished observations) do

Table 2 Mean rate of atrial tension change over 30 s of electrically-stimulated auricles excised from groups of Sprague-Dawley, fatty, Zucker (fa/fa) and lean, hooded (Fa/fa) rats following administration of noradrenaline, isoprenaline or carbachol alone or plus one of the pentapeptides

Noradrenaline	*NA*	*NA + ME*	*NA + LE*	*NA + DAMEA*	*NA + DALEA*
Sprague-Dawley	2.5 ± 0.4	3.0 ± 0.4	2.7 ± 0.4	3.7 ± 0.3	3.1 ± 0.5
Fa/fa	5.0 ± 0.6*	5.2 ± 0.3	5.5 ± 0.3	5.3 ± 0.3	5.3 ± 0.4
fa/fa	3.5 ± 0.3	3.6 ± 0.7	3.7 ± 0.5	3.7 ± 1.8	3.8 ± 0.9
Isoprenaline	*Iso*	*Iso + ME*	*Iso + LE*	*Iso + DAMEA*	*Iso + DALEA*
Sprague-Dawley	4.9 ± 1.5	3.2 ± 1.5	3.6 ± 0.5	4.0 ± 0.4	4.2 ± 0.1
Fa/fa	8.3 ± 0.9**	6.2 ± 1.2	6.7 ± 1.8	5.0 ± 1.8	5.6 ± 2.3
fa/fa	5.6 ± 1.4	6.1 ± 1.5	6.6 ± 1.6	6.5 ± 2.0	3.7 ± 1.4
Carbachol	*Carb*	*Carb + ME*	*Carb + LE*	*Carb + DAMEA*	*Carb + DALEA*
Sprague-Dawley	−2.0 ± 0.2	−1.5 ± 0.4	−1.8 ± 0.2	−2.2 ± 0.7	−2.2 ± 0.5
Fa/fa	−2.7 ± 0.9	−1.4 ± 0.5	−2.1 ± 0.3	−1.8 ± 0.6	−1.9 ± 0.4
fa/fa	−1.9 ± 0.6	−2.8 ± 1.1	−3.0 ± 0.6	−2.6 ± 0.7	−1.9 ± 0.6

Values show mean change in mg ± s.e.mean. Abbreviations: NA, noradrenaline HCl (10^{-6}M) final bath concentration; Iso, isoprenaline HCl (10^{-6}M) final bath concentration; Carb, carbamylcholine chloride (10^{-8}M) final bath concentration; ME, methionine enkephalin; LE, leucine enkephalin; DAMEA, [D-Ala^2-methionine] enkephalinamide; DALEA, [D-Ala^2-leucine] enkephalinamide, all 4×10^{-6} M final bath concentration.
* $P < 0.05$, ** $P < 0.01$, significantly different from rate of atrial tension development in Sprague-Dawley and fa/fa rats.

not, with the doses tested (4×10^{-7} to 4×10^{-4}M), cause any changes in atrial tension of electrically-stimulated auricles excised from either lean or obese rats. This result is consistent with the findings of Koyama *et al.* (1984) who obtained no evidence of a direct cardiac action for [Leu] enkephalin in a canine heart-lung preparation. That study further suggested that the central nervous system is probably involved in mediating the cardiovascular actions of [Leu] enkephalin. However, direct effects of [Leu] and [Met] enkephalin on the peripheral vasculature cannot be ruled out (Sander *et al.*, 1981).

Eiden & Ruth (1982) indicated that [Met] and [Leu] enkephalin, β-endorphin and other pentapeptide derivatives had no effect on the basal beating rate of isolated atria of Sprague-Dawley rats. Yet, [Met] enkephalin (10^{-7}M) reduced the maximum chronotropic action of noradrenaline by 40%, an inhibitory effect that was readily blocked by pretreatment with naloxone (10^{-7}M). In our study, none of the four pentapeptides tested (4×10^{-6}M) modulated either the positive inotropic actions of NA or isoprenaline or the negative inotropic effects of carbachol in any atria isolated from three strains of rats.

It is interesting to note that Robberecht *et al.* (1983) found that secretin and (±)-isoprenaline were equipotent in increasing tension of the papillary muscle of the right ventricle of lean rats (Fa/fa). With age-matched obese rats (fa/fa) tension development of both drugs was reduced compared to that seen in the lean group and secretin had a much weaker inotropic effect than isoprenaline. In the present study, NA and isoprenaline caused the greatest increase and fastest rate change in atrial tension in the lean, hooded heterozygous group (Fa/fa). Whether these variations in potency of NA and isoprenaline between the strains of rats tested suggests differences in the number of α- and/or β-adrenoceptors on the myocardium, that produce the positive inotropic actions to NA and isoprenaline (Farah *et al.*, 1984), in these groups is only speculation. Carbachol, which has been shown previously (Vadlamudi & McNeill, 1983) to reduce ventricular pressure development in both control and diabetic rat hearts, in this study was more potent, on a molar basis, as a negative inotropic agent on atria isolated from both lean and obese rats than either NA or isoprenaline was at producing positive inotropic effects.

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