J. Pharm. Pharmacol. 1988, 40: 648–650
Communicated March 8, 1988

Effects of proenkephalin products on rat cardiac and vascular tissue in-vitro

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Abstract—The cardiovascular effects of the four proenkephalin products [Met]-enkephalin (ME), [Leu]-enkephalin (LE), [Met]-enkephalyl-arg^{6}phe^7 (MEAP) and [Met]-enkephalyl-arg^6-gly^7-leu^8 (MEAGL) have been studied on the isolated spontaneously beating rat atria and the perfused rat mesentery in-vitro. All four peptides (at concentrations up to 10^{-6}M and in the presence of peptidase inhibitors) had no direct effect on atrial rate or contractility and did not alter responses to noradrenaline or field stimulation. In addition, the peptides had no effect on the perfusion pressure of the mesentery and did not alter vasoconstrictor responses to noradrenaline. The results show that proenkephalin products are without direct or modulating effects on atrial muscle or mesenteric vasculature of the rat, and suggest that there is no endogenous opioid control in these tissues.

Cloning and sequencing of the proenkephalin gene has indicated that processing of the precursor protein yields seven enkephalin sequences; four copies of [Met]-enkephalin, and one copy each of [Leu]-enkephalin, [Met]enkephalyl-arg^{6}phe^7 and [Met]-enkephalyl-arg^6-gly^7-leu^8 (Gubler et al 1982; Noda et al 1982). High concentrations of proenkephalin derived peptides are found in the adrenal medulla where they are co-stored with catecholamines and splanchnic nerve stimulation results in the release of the contents of the chromaffin granules into the adrenal vein. In addition, the presence of enkephalins and opioid receptors in peripheral tissues such as the heart, sympathetic ganglia and vascular smooth muscle has raised the question as to whether circulating enkephalins may play a role in the peripheral regulation of the cardiovascular system (see Holaday 1983).

There is evidence that the opioid pentapeptides may have direct or indirect effects on cardiac and vascular tissue in the rat, though there are conflicting reports in the literature (see Douglas & Kitchen 1988). Accordingly, we have studied the effects of all four proenkephalin peptides for both direct actions upon cardiac and mesenteric vascular tissue in-vitro, and for any modulatory effects upon responses to noradrenaline or field stimulation.

Materials and methods

Isolated rat atria. Male Wistar albino rats (University of Surrey strain, 200–250g) were decapitated and the hearts quickly excised and immersed in ice-cold Krebs-Ringer solution. The atria were dissected and mounted in an organ bath at 30°C. A resting tension of 500 mg was applied to the tissue which was aerated with 95% O_2/5% CO_2 and allowed to equilibrate for 75 min. Atrial force and integrated rate were recorded isometrically using a Grass FT03 transducer linked to a Grass Model 79D polygraph. Chronotropic and inotropic responses to noradrenaline were measured using intermittent dose-response curves with 4 min between drug additions. For study of noradrenergic and cholinergic nerve stimulation, field stimulation responses were obtained in the presence of 1 μM atropine or 1 μM propranolol, respectively. Field stimulation across linear platinum electrodes (0·3 mm dia. × 6·5 cm length, 0·5 cm apart) was provided by trains of square wave pulses from a Grass S88 stimulator (4 s duration, 40V, 1–10 Hz pulses of 2 ms width every 100 s).

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The effects of each proenkephalin product upon responses to noradrenaline or field stimulation were studied in separate experiments. Atrial tissues were preincubated with peptidase inhibitors (Bestatin, 10 μM, Captopril, 10μM, and Leucyl-leucine, 2 mM) for 30 min and control dose-response curves to field stimulation were carried out followed by incubation with peptide for 15 min. On each preparation one peptide was studied for its effect on noradrenaline or field stimulation using four increasing concentrations of peptide. A 45 min period was allowed between each response curve. For noradrenaline responses, EC50 values for noradrenaline (concentration producing 50% of maximum response) were calculated for each individual experiment using linear regression. For noradrenergic field stimulation, EF10 values were determined (defined as the % of maximum chronotropic response evoked by 10 Hz stimulation) and for cholinergic field stimulation, EF2·5 values were determined (defined as the % maximum chronotropic response evoked by 2·5 Hz stimulation).

Isolated perfused rat mesentery. Male Wistar albino rats (University of Surrey strain, 250–300 g) were anaesthetized using sodium pentobarbitone (60 mg kg^{-1} i.p.). The abdomen was opened, the superior mesenteric artery cannulated and the vascular supply to the mesentery removed as described by McGregor (1965). The mesentery was perfused with aerated (95% O_2/5% CO_2) Krebs-Ringer at a rate of 2–4 mL min^{-1} and maintained at 37°C. Preparations were allowed to equilibrate for 60 min before drug additions. Perfusion pressure was measured using an in-line pressure transducer (Statham P231D) linked to a Grass Model 79D polygraph. Drugs were administered via a 3-way tap close to the point of cannulation. As for atrial studies control dose response curves to noradrenaline were obtained using a 4 min dose cycle with peptidase inhibitors added to the perfusate. Dose response curves were repeated at 45 min intervals preceded by a 15 min perfusion with a proenkephalin peptide. For each preparation four increasing concentrations of peptide were studied for modulation of noradrenaline responses.

Drugs. ME, LE, MEAP and MEAGL were purchased from Cambridge Research Biochemicals. Bestatin and leucyl-leucine were purchased from Sigma. Captopril and naloxone were gifts from Squibb and Dupont, respectively.

Results

ME, LE, MEAP and MEAGL (10^{-9}–10^{-6}M) and naloxone (10^{-6}M) or peptidase inhibitors alone had no effect on basal rate or force of contraction. Incubation of atria with ME, LE, MEAP or MEAGL (10^{-9}–10^{-6}M) in the presence of peptide inhibitors had no significant effect on inotropic responses induced by noradrenaline (5×10^{-9}–2×10^{-7}M) (Table 1). There were indications of a small enhancement of chronotropic responses to noradrenaline in the presence of LE and MEAP although there was no clear dose relationship (Table 1).

Incubation of atria with ME, LE, MEAP and MEAGL (10^{-9}–10^{-6}M) in the presence of peptidase inhibitors or naloxone

Table 1. Chronotropic (A) and inotropic (B) responses of rat atria to noradrenaline in the presence and absence of proenkephalin products.

		ΔEC50 values for noradrenaline (nM) Peptide concentration			
Peptide		10^{-9}	10^{-8}	10^{-7}	10^{-6}
A	ME	+3·1±5·3	−6·2±4·0	−0·1±4·4	−5·1±3·1
	LE	+7·0±6·7	−7·1±6·7	−8·4±7·0	−10·3±6·6
	MEAP	+1·7±6·7	−13·1±5·0	−15·1±4·9*	−6·7±4·2
	MEAGL	+6·2±5·8	−2·0±4·3	−1·2±1·3	−2·4±4·9
B	ME	−3·9±3·2	+0·9±2·5	−1·8±1·3	+0·7±0·2
	LE	+1·6±3·9	+8·4±5·5	+0·8±4·7	+1·6±4·7
	MEAP	−3·6±3·6	+2·1±6·1	+4·4±4·4	+3·2±5·1
	MEAGL	+6·9±4·4	+5·1±4·7	+8·4±4·5	+5·9±6·6

Values represent the mean change ± s.e.m. in EC50 for noradrenaline for 5 paired determinations. The mean basal rate was 190 ± 8 b min^{-1} (n = 16), the maximum chronotropic response to noradrenaline was +89 ± 7 b min^{-1} (n = 16) and the maximum inotropic response was +310 ± 11 mg (n = 16). Paired *t*-test; * $P < 0.05$.

(10^{-6}M) had no effect on the chronotropic response induced by noradrenergic or cholinergic nerve stimulation (Table 2).

ME, LE, MEAP or MEAGL (10^{-9}–10^{-6}M) or perfusion with peptidase inhibitors alone had no effect on mesentery perfusion pressure. In addition, the ED50 values for noradrenaline was not significantly altered by any of the proenkephalin products (Table 3). Responses to nerve stimulation were not sufficiently consistent to allow study of modulatory effects of the peptides.

Table 2. Chronotropic responses to noradrenergic (A) and cholinergic (B) field stimulation in the presence and absence of proenkephalin products.

		Peptide concentration				
Peptide		—	10^{-9}	10^{-8}	10^{-7}	10^{-6}
		EF10 (% Maximum response)				
A	ME	74±3·6	66±5·2	72±3·9	65±6·1	71±4·9
	LE	65±7·2	66±5·1	74±4·3	78±5·2	63±6·7
	MEAP	72±5·2	68±3·7	63±7·2	72±4·9	75±5·3
	MEAGL	75±2·9	71±5·8	68±5·7	70±4·4	67±5·9
		EF2·5 (% Maximum response)				
B	ME	65±6·7	57±5·7	68±4·4	63±4·8	59±3·9
	LE	59±7·1	64±4·8	62±5·2	67±5·2	59±5·0
	MEAP	64±4·5	69±4·3	67±6·1	60±4·4	60±5·5
	MEAGL	64±5·3	62±5·3	58±3·9	52±6·3	66±4·7

Values are the mean ± s.e.m. of 4 determinations. The maximum response was +79 ± 9 b min^{-1} (n = 16) for noradrenergic stimulation and −41 ± 9 b min^{-1} (n = 16) for cholinergic stimulation. Maximum cholinergic and noradrenergic responses were observed at 4–6Hz and 15–20Hz, respectively. Absolute data was transposed to percentage of maximum response against stimulation frequency. EF10 and EF2·5 values calculated as described in Methods. One-way analysis of variance for each individual peptide versus noradrenergic or cholinergic field stimulation responses—no significant differences.

Table 3. Pressor responses of the perfused rat mesentery to noradrenaline in the presence and absence of proenkephalin products

	ED50 values for noradrenaline (nmol) Peptide concentration (M)				
Peptide	—	10^{-9}	10^{-8}	10^{-7}	10^{-6}
ME	6·7±0·7	7·6±0·9	5·7±0·7	7·3±1·1	6·0±0·7
LE	8·1±1·1	8·3±1·2	8·5±1·2	7·2±0·7	7·5±1·3
MEAP	9·2±0·7	9·6±1·2	8·8±1·4	10·2±0·8	9·1±1·0
MEAGL	6·5±1·2	5·7±1·2	5·0±0·5	5·7±0·7	4·4±0·7

Values are the mean ± s.e.m. of 4 determinations. Peptide concentrations refer to the levels in the perfusate. The maximum pressor response to noradrenaline was 75 ± 8 mm Hg (n = 16). One-way analysis of variance for each individual peptide versus pressor responses—no significant differences.

Discussion

In common with previous studies with the pentapeptides ME and LE (13–16) no direct effects upon atrial rate or contractility were observed. We were also unable to show any modulating effects on inotropic responses to noradrenaline which contrasts with earlier studies (Eiden & Ruth 1982) but is in agreement with more recent work which failed to observe effects of proenkephalin products upon inotropic responses to noradrenaline (Saunders & Thornhill 1985; Parker et al 1986; Willerth & Thornhill 1987). Eiden & Ruth (1982) also observed a reduced chronotropic response to noradrenaline in the presence of the opioid pentapeptides. There was no indication of such effects in our study; indeed LE and MEAP produced a slight potentiation of chronotropic responses to noradrenaline, and ME and MEAGL were without effect. In addition a lack of modulatory effect of several proenkephalin derived peptides on atrial responses to adrenaline has also been reported (Cherdchu et al 1987). Previous studies have reported an inhibition of cholinergic but not noradrenergic responses in rat atria by structural analogues of the enkephalins (Wong-Dusting & Rand 1985). In contrast we failed to observe modulating effects for all four proenkephalin products on either cholinergic or noradrenergic nerve stimulation and it is unlikely that our negative results are due to metabolic inactivation as peptidase inhibitors were used in all experiments. The question of existence of modulating presynaptic opioid receptors therefore remains open but it should be stressed that the receptor sensitivities of enkephalin analogues are dissimilar from the endogenous peptides (Paterson et al 1983).

Though actions of the opioid pentapeptides have been reported in other vascular tissues of the rat (Ruth et al 1984; Yamamoto et al 1984; Illes et al 1987), in our study all four proenkephalin products were without effect in the mesenteric vasculature. Thus, despite microscopic evidence for dilation of mesenteric terminal arterioles by opioids (Altura et al 1978) this may not have relevance to the total perfusion pressure in this bed.

In conclusion, the lack of effect of opioid products of proenkephalin in both atrial and mesenteric beds, even in the presence of peptidase inhibitors, militate against endogenous opioid control in these tissues.

We are grateful to Dupont and Squibb for the gift of drugs. H.D. is supported by an S.E.R.C. Scholarship.

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J. Pharm. Pharmacol. 1988, 40: 650–651
Communicated February 9, 1988

Effect of tiopronin on prostaglandin synthesis in rabbit kidney medulla slices

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Abstract—The effect of 2-mercaptopropionylglycine (tiopronin), which is widely used for the treatment of various hepatic disorders, on the generation of medullary prostaglandins (PG) E_2 and $F_{2\alpha}$ has been examined. Tiopronin had a potent inhibitory effect on PG E_2 formation. Simultaneously, PG $F_{2\alpha}$ production was increased. In the presence of tiopronin the net increased amount of PG $F_{2\alpha}$ was much smaller than the net decreased amount of PG E_2 (6–20%). These results suggest that tiopronin has the potential to modulate PG E_2 and $F_{2\alpha}$ synthesis by affecting endoperoxide E_2 isomerase or endoperoxide reductase and that this effect may represent some pharmacological action of the drug.

It has been reported that 2-mercaptopropionylglycine (tiopronin), a sulphhydryl compound, reduces the hepatotoxicity of paracetamol or carbon tetrachloride (Labadarios et al 1977; Horiuchi et al 1979). Thus, it is widely used for the treatment of various hepatic disorders. The clinical course of patients with liver cirrhosis is frequently complicated by progressive impairment of renal sodium handling (Epstein 1979). The renal medulla is rich in prostaglandins (PGs) as well as in the enzymes that biosynthesize them. Intrarenal PGs, seem to be determinants of renal haemodynamics and renal sodium handling in both normal and cirrhotic man (Epstein et al 1982). Recently, we have reported that sulphhydryl compounds, such as reduced glutathione and cysteine, play a role in the control of PG E_2 and $F_{2\alpha}$ synthesis in renal medulla (Fujita et al 1986). Those findings prompted us to examine the effect of tiopronin on the in-vitro generation of medullary PGE_2 and $F_{2\alpha}$.

Materials and methods

Male rabbits (2–2·5 kg wt.) were used. The kidneys were removed from anaesthetized (sodium pentobarbitone, 30 mg kg^{-1}) rabbits and rapidly chilled in ice-cold 0·9% NaCl (saline). The kidney medulla slices were prepared as previously described (Fujimoto & Fujita 1982). In all experiments, the slices (0·4 g) were preincubated in 4·0 mL 0·15 M KCl/0·02 M Tris HCl buffer (pH 7·4) at 4°C for 5 min. After preincubation, the medium was discarded, the slices rinsed twice with the Tris HCl buffer and incubated with the indicated concentrations of tiopronin (Santen Pharmaceuticals Ltd, Japan) at 37°C for 30 min.

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We reported previously that the major PGs produced in our incubation of medulla slices and recovered in the medium were E_2 and $F_{2\alpha}$ (Fujimoto et al 1983). PG E_2 and $F_{2\alpha}$ in the incubation medium were simultaneously determined by a high-pressure liquid chromatographic (HPLC) method as described by Fujita et al (1986). Briefly, PG E_2 and $F_{2\alpha}$ extracted with ethyl ether (approximately pH 3) were measured after esterification of the PGs with 9-anthryldiazomethane (ADAM) (Nimura & Kinoshita 1980). Since ADAM contains many impurities which interfere with the HPLC determination, the purification of PGs esterified with ADAM (PGs-ADAM) was attempted using a normal-phase silica cartridge (Sep-pak, Waters Associates). The cartridge was prepared by rinsing it with 5 mL of methanol followed by 10 mL of benzene–ethyl acetate (60:40 v/v). The sample was passed through the cartridge. The cartridge was washed with benzene–ethyl acetate (60:40 v/v, 7 mL) and the PGs-ADAM was then quantitatively eluted with benzene–ethyl acetate–methanol (60:40:5 v/v, 7 mL). Peak heights were measured for the quantification of the PGs-ADAM relative to the standard derivatives prepared from authentic PG E_2 and $F_{2\alpha}$.

The values presented herein are the means ± s.e.m. Statistical significance was calculated using Student's paired *t*-test.

Results

Fig. 1 illustrates the effects of various concentrations of

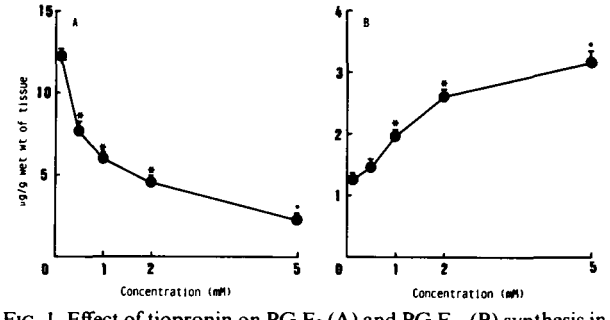

FIG. 1. Effect of tiopronin on PG E_2 (A) and PG $F_{2\alpha}$ (B) synthesis in rabbit kidney medulla slices. Slices were incubated for 30 min at 37°C in 0·15 M KCl/0·02 M Tris HCl buffer in the presence of different concentrations of tiopronin. Each point indicates the mean of 5 experiments; vertical lines show s.e.m. $*P < 0·01$ compared with the corresponding value in the absence of tiopronin.