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Altered neuropeptide Y Y_1 responses in mesenteric arteries in rats with congestive heart failure

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Abstract

The aim of the present study was to elucidate if the potentiating effect of neuropeptide Y on various vasoactive agents in vitro is (1) altered in mesenteric arteries from rats with congestive heart failure and (2) mediated by the neuropeptide Y Y_1 receptor. The direct vascular effects of neuropeptide Y and its modulating effects on the contractions induced by endothelin-1-, noradrenaline-, 5-hydroxytryptamine (5-HT)-, U46619-(9, 11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$) and ATP, and acetylcholine-induced dilatations were studied in the presence and absence of the neuropeptide Y Y_1 antagonist, BIBP3226 (BIBP3226{(*R*)-*N*2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-D-arginine-amide}). Neuropeptide Y, per se, had no vasoactive effect in the arteries. The potency of endothelin-1 was significantly decreased in congestive heart failure rats. Neuropeptide Y and neuropeptide Y-(13–36) potentiated the endothelin-1-induced contraction in congestive heart failure mesenteric arteries. In 20% of the congestive heart failure rats, sarafotoxin 6c induced a contraction of 31 ± 4%. Neuropeptide Y also potentiated U46619- and noradrenaline-induced contractions but not 5-HT-induced contractions in congestive heart failure arteries. In sham-operated animals neuropeptide Y potentiated noradrenaline- and 5-HT-induced contractions. These potentiations were inhibited by BIBP3226. Acetylcholine induced an equipotent relaxation in both groups which was unaffected by neuropeptide Y. In conclusion, neuropeptide Y responses are altered in congestive heart failure rats. The potentiating effect differs between vasoactive substances. Neuropeptide Y Y_1 and non-neuropeptide Y_1 receptors are involved.

Keywords: Congestive heart failure; Mesenteric artery; Neuropeptide Y Y_1 receptor; Potentiation; (Rat); BIBP3226

1. Introduction

Neuropeptide Y is a 36-amino-acid peptide which is co-stored with noradrenaline in postganglionic nerves supplying the cardiovascular system. It is released with noradrenaline on sympathetic nerve stimulation. Neuropeptide Y contracts blood vessels and increases blood pressure mainly through the postjunctional neuropeptide Y Y_1 receptor (Grundemar and Håkanson, 1993). Activation of the prejunctional neuropeptide Y Y_2 receptor has been shown to inhibit the presynaptic release of both noradrenaline and neuropeptide Y. However, there is evidence for neuropeptide Y Y_2 receptor-mediated vasoconstriction in, e.g., the splenic vascular bed of the pig (Modin et al., 1991) and a neuropeptide Y Y_2 receptor-mediated inhibition of the acetylcholine-induced relaxation in guinea pig basilar arteries (Nilsson et al., 1996c).

While neuropeptide Y potently increases mean arterial pressure and vascular resistance in vivo in both animals (Wahlestedt et al., 1990) and in humans (Clarke et al., 1987), the direct contractile effect in rat vessels in vitro is weak or absent (Andriantsitohaina and Stoclet, 1988; Grundemar and Högestätt, 1992). One major action of neuropeptide Y appears to be to potentiate, at subthreshold concentrations, the contractile response to nerve stimulation and to a variety of vasoactive agonists (Edvinsson et al., 1984; Ekblad et al., 1984). This effect, thought to be mediated by the neuropeptide Y Y_1 receptor, has been demonstrated both in vitro in vascular preparations and in vivo in pithed and in conscious rats (Andriantsitohaina and Stoclet, 1988; Sun et al., 1992). The neuropeptide Y receptors and their functions were first characterised using truncated neuropeptide Y receptor analogues (Wahlestedt et al., 1986). A turning point for receptor characterisation

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as well as for the understanding of the role of neuropeptide Y came with the introduction of competitive, specific and selective neuropeptide Y Y_1 receptor antagonists, BIBP3226, SR120107A and SR120819A, which all have proven to be antagonists with nanomolar affinity for the neuropeptide Y Y_1 receptor in several species, including the human (Serradeil-Le Gal et al., 1995; Doods et al., 1996; Malmström and Lundberg, 1996).

Congestive heart failure is a clinical syndrome characterised by impaired cardiac performance, decreased cardiac output and increased peripheral resistance due to several disturbances of neurohumoral regulation (Francis et al., 1984). Elevated levels of noradrenaline and neuropeptide Y, among several vasoactive hormones, have been reported, being due to enhanced sympathetic nervous activity (Leimbach et al., 1986; Maisel et al., 1989). These neurohumoral disturbances are claimed to be of major importance for the progression and pathogenesis of congestive heart failure, and elevated plasma levels of neuropeptide Y and noradrenaline have been shown to correlate with the severity of congestive heart failure (Edvinsson et al., 1990).

It is logical to assume that there are changes in sympathetic receptor characteristics, i.e., sensitivity and activity, since prolonged exposure to an agonist generally results in 'downregulation' of receptor binding sites and function. Thus decreased vascular responses, along with elevated levels of the corresponding peptide, have been found for α-adrenoceptors and endothelin ET_A receptors in rats with experimental congestive heart failure (Feng et al., 1996; Sakai et al., 1996).

In the present study, we ligated the left coronary artery in the rat, resulting in myocardial infarction with the subsequent development of congestive heart failure. This is a well-established animal model with pathophysiological alterations similar to those seen in ischemic heart disease in man (Pfeffer et al., 1979; Bergdahl et al., 1995). The mesenteric artery was chosen because it is densely innervated by sympathetic nerves and is hence suitable for showing effects of sympathetic peptides (Edvinsson et al., 1985). Since direct contractile responses to neuropeptide Y are difficult to obtain in vitro (Grundemar and Högestätt, 1992) the potentiating effect of neuropeptide Y was investigated with the aim to elucidate if the response is altered in congestive heart failure and if the potentiation is mediated by the neuropeptide Y Y_1 receptor.

2. Materials and methods

2.1. Induction of myocardial infarction

Male Sprague–Dawley rats (ALAB; Sweden) were used. During intraperitoneal anaesthesia (Mebumal® 50 mg kg^{-1}; ACO, Sweden) the rats were intubated and artificially ventilated with a respirator for small animals, developed by the Department for Technological and Medical Service at Lund University Hospital. A left thoracotomy was performed, exposing the left ventricular wall. The left coronary artery was ligated by positioning a suture between the pulmonary artery out-flow tract and the left atrium. The lungs were hyperinflated thereafter using positive end-expiratory pressure and the thorax was closed immediately. The rats were allowed to recover for at least 4 weeks before in vitro experiments started. In parallel, some rats were subjected to the same surgical procedure but without coronary ligation (sham). These sham-operated rats served as controls and did not differ from non-operated rat.

2.2. Evaluation of infarct size

To confirm the presence of myocardial infarction in the operated animals a histological study of the infarcted hearts was performed. During the in vitro preparation (see below) the hearts from the sham and the congestive heart failure rats were removed and immersed in a 6% formaldehyde solution. The ventricular region of the heart was then cut from the apex to the base in four transverse slices and 10 μm thin sections from each slice were stained and prepared for light microscopic evaluation. Photographs were made at 10-times magnification. The endocardial circumference of the left ventricle and the extent of the fibrotic area from each slice was measured. Infarct size was evaluated according to Pfeffer et al. (1979); the fibrotic fraction of the total cross-sectional endocardial circumference of the left ventricle was measured. Earlier studies of the congestive heart failure rat model have shown that the impairment of left ventricular function is directly related to the loss of myocardium (Pfeffer et al., 1979). Subsequently, a loss of more than 30% of the myocardium of the left ventricle results in pump dysfunction with reduced values of peak flow, systolic and mean arterial pressure.

2.3. In vitro preparation

The animals (weighing 250–350 g) were anaesthetized with CO_2 and killed by a cut through the heart. The superior mesenteric artery with adherent branches was immediately taken out and immersed in a chilled (4°C) buffer solution aerated with 5% CO_2 in O_2. The buffer solution was composed of the following substances (in mM): NaCl 119; $NaHCO_3$ 15; KCl 4.6; $MgCl_2$ 1.2; NaH_2PO_4 1.2; $CaCl_2$ 1.5; glucose 5.5. The first order of the arterial branches was carefully dissected free from adhesive tissue under an operating microscope and cut into ring segments with a length of 2–3 mm. The segments were immersed in temperature-controlled tissue baths with a volume of 2.5 ml containing the above described buffer solution and continuously aerated with 5% CO_2 in O_2 to maintain a pH of 7.4. Each vessel segment was carefully mounted on two L-shaped metal prongs (0.1 mm in diame-

Table 1
The potentiating effect of neuropeptide Y on the endothelin-1-induced contraction in the presence and abscense of BIBP3226 or FR139317 in sham and congestive heart failure rat mesenteric arteries

	n	pEC50	E_{max}
Sham ET-1	9	8.5 ± 0.1	97 ± 6
Sham ET-1 + NPY	9	8.4 ± 0.1	104 ± 9
CHF ET-1	11	8.2 ± 0.1[a]	99 ± 3
CHF ET-1 + NPY	11	8.6 ± 0.1[b]	97 ± 5
CHF ET-1 + NPY + BIBP3226	8	8.7 ± 0.1[c]	98 ± 5
CHF ET-1 + NPY + FR139317	6	7.6 ± 0.1[c]	91 ± 6
CHF ET-1 + NPY-(13–36)	6	8.9 ± 0.2[c]	98 ± 5

Values represent mean ± standard error of the mean. *n* refers to the number of rats from which the mesenteric arteries were collected, pEC_{50} refers to the negative logarithm of the molar concentration of agonist inducing a half-maximum response, E_{max} refers to the maximum effect on contraction in percent of potassium-induced contraction, ET-1 denotes endothelin-1, CHF denotes congestive heart failure and NPY denotes neuropeptide Y.
[a] $P < 0.05$ CHF vs. sham rats (Mann–Withney *U*-test); [b] $P < 0.01$; [c] $P < 0.05$ vs. ET-1 alone (Wilcoxon signed-rank test).

ter). One prong was connected to a force-displacement transducer (FTO3C, Grass Instr., Quincy, USA) attached to a MacLab unit for continuous recording of isometric tension using the Chart™ software (ADI Instruments, Hastings, UK). The other prong was connected to a displacement device, allowing fine adjustments of the distance between the two parallel prongs. A passive tension of 2–3 mN was applied to the segments and they were allowed to stabilise at this tension for 1 h.

The contractile capacity of each vessel segment was examined by exposure to a K^+-rich (60 mM) buffer solution which had the same composition as the standard solution except that NaCl was exchanged for an equimolar amount of KCl. Arteries were included when repeated exposure to the K^+-rich solution induced a contractile response with less than 10% variation. After each K^+-induced contraction the vessels were allowed to stabilise at resting tension by means of repeated washing with the standard solution.

Direct contraction was studied by adding agonists in a concentration–response cumulative fashion, where a higher concentration was added at the maximum effect of the preceding concentration. Neuropeptide Y receptor-induced potentiation was studied by adding neuropeptide Y (10 nM) 2 min before adding the agonist (endothelin-1, sarafotoxin 6c, noradrenaline, 5-HT, U46619 or ATP). Various concentrations of neuropeptide Y were initially used and the one used in the study was the first concentration to give a reproducible and strong potentiation. The controls were an equal number of matched vascular segments run in parallel. These arteries received endothelin-1, noradrenaline, 5-HT, U46619 or ATP without prior exposure to neuropeptide Y. The neuropeptide Y receptor antagonist BIBP3226 (1 μM) was added in turn 15 min before neuropeptide Y. In part of the endothelin experiments (see Section 3) the neuropeptide Y Y_2 receptor agonist, neuropeptide Y-(13–36), was used in the same way and at the same concentration (10 nM) as described for neuropeptide Y.

In order to study relaxant activity the arterial segments were precontracted with 30 nM U46619 (a concentration above the pEC_{50} value), causing a contraction which remained stable for 45–60 min. in the control experiments. When the precontraction was stable, direct relaxation was studied by adding neuropeptide Y or acetylcholine in a cumulative fashion. To study the inhibition of the acetylcholine-induced relaxation, neuropeptide Y (10 nM), was added before precontraction.

The results are given as percentages of the K^+-induced contraction or as percentages of the U46619-induced contraction (relaxation experiments). The maximum effect, contraction or relaxation, is given as E_{max}. The potency of the drugs is expressed as pEC_{50} (the negative logarithm of the molar concentration of the drug inducing a half-maximum response). The data are shown as means ± standard error of the mean; *n* refers to the number of rats used.

2.4. Drugs

The following drugs were used: neuropeptide Y (rat), neuropeptide Y-(13–36), endothelin-1, sarafotoxin 6c

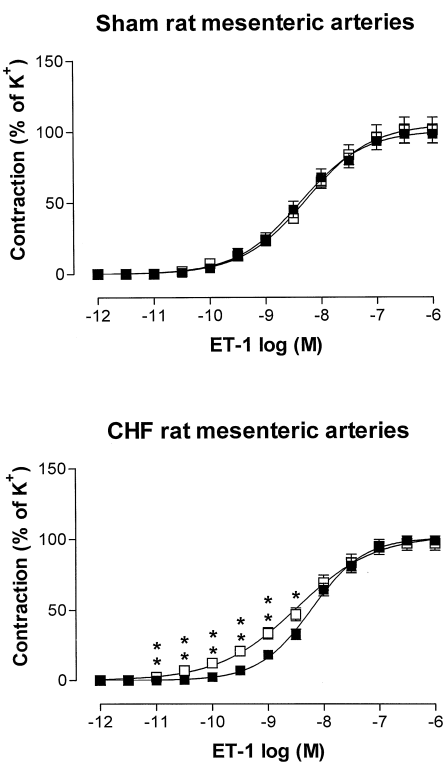

Fig. 1. The contractile response to endothelin-1 in the absence (■) and presence (□) of neuropeptide Y (10 nM) in sham and congestive heart failure rat mesenteric arteries. The results are expressed as percentages of the K^+-induced contraction, each point represents mean ± standard error of the mean of 9–11 experiments. * $P < 0.05$, ** $P < 0.01$ (Wilcoxon signed-rank test).

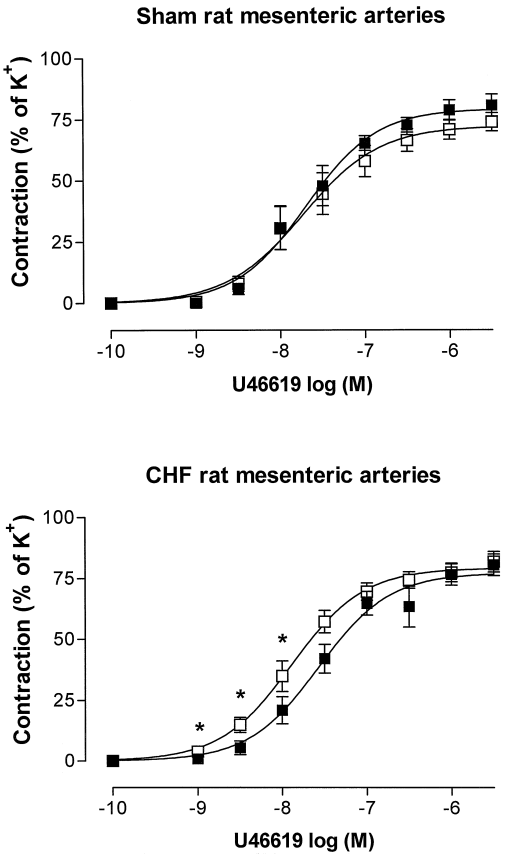

Fig. 2. The contractile response to U46619 in the absence (■) and presence (□) of neuropeptide Y (10 nM) in sham and congestive heart failure rat mesenteric arteries. The results are expressed as percentages of the K^+-induced contraction, each point represents mean ± standard error of the mean of 6–10 experiments. * $P < 0.05$ (Wilcoxon signed-rank test).

(Auspep, Parkville, Australia), noradrenaline, U46619 (9, 11-dideoxy-11α, 9α-epoxymethano-prostaglandin $F_{2\alpha}$), adenosine 5′-triphosphate (ATP), acetylcholinchloride (acetylcholine) and 5-hydroxytryptamine (5-HT) (Sigma, St. Louis, MO, USA), FR139317 ((*R*)2-[(r)-2-[[1-(hexahydro-1*H*-azepinyl9]-carbonyl]amino-4-methyl-pentanoyl] amino-3-[3-(1-methyl-1*H*-indolyl)]propionyl]amino-3-(2-pyridyl)propionic acid) (Fujisawa Pharmaceuticals, Osaka, Japan) and BIBP3226 (BIBP3226 {(*R*)-*N*2-(diphenylacetyl)-*N*-[(4-hydroxyphenyl)methyl]-D-arginine-amide}) a generous gift from Dr. H. Doods, Dr. Karl Thomae, Germany.

The drugs were dissolved in 0.9% sterile NaCl with 0.1% bovine serum albumin (Sigma, St. Louis, MO, USA), (neuropeptide Y, endothelin-1, sarafotoxin 6c, ATP and acetylcholine). Noradrenaline and 5-HT were dissolved in sterile 0.9% NaCl with ascorbic acid (0.1 mM) added to avoid oxidation. U46619 and FR139317 were dissolved in ethanol 70% stored in tubes as stock solution of 0.1 mM and kept in a freezer (−70°). The stock solutions were diluted in 0.9% NaCl to 1 μM just before use.

2.5. Statistics

Statistical significance was determined with the Mann–Whitney *U*-test (for unpaired observations), Wilcoxon signed-rank test (for paired observations) and Student's *t*-test were used for comparison of K^+- and U46619-induced contractions. The StatView II™ software was used on a Macintosh IIcx computer. Values including neuropeptide Y/neuropeptide Y + BIBP3226 are compared to the dose–response curves for the specific contractile or dilatory agonist respectively, $P < 0.05$ was considered significant.

The study was approved by the Committee of Ethics for Animal Experiments at the University of Gothenburg and the University of Lund, Sweden.

3. Results

3.1. Confirmation of congestive heart failure

The operated rats exhibited, when opened, signs of congestive heart failure such as abdominal ascites and pulmonary oedema. The hearts showed post-infarction signs comprising fibrosis and enlargement of the left ven-

Table 2
The potentiating effect of neuropeptide Y (NPY) on the contractile responses to noradrenaline (NA), 5-hyroxytryptamine (5-HT), U46619 and adenosine 5′triphosphate (ATP) in the presence and absence of BIBP3226 in sham and congestive heart failure (CHF) rat mesenteric arteries

	NA			5-HT			U46619			ATP		
	n	pEC_{50}	E_{max}	*n*	pEC_{50}	E_{max}	*n*	pEC_{50}	E_{max}	*n*	pEC_{50}	E_{max}
Sham (substance[a])	10	5.8 ± 0.1	116 ± 8	9	6.8 ± 0.2	106 ± 3	6	7.8 ± 0.2	81 ± 5	8	4.5 ± 0.2	44 ± 7
Sham (substance + NPY)	10	6.2 ± 0.1[b]	111 ± 3	9	7.3 ± 0.2[b]	104 ± 5	6	7.8 ± 0.2	74 ± 4	8	4.4 ± 0.2	45 ± 7
Sham (substance + NPY + BIBP3226)	5	5.7 ± 0.2	121 ± 5	5	6.7 ± 0.3	105 ± 2	–	–	–	–	–	–
CHF (substance)	10	6.0 ± 0.1	114 ± 3	11	6.7 ± 0.1	116 ± 9	10	7.6 ± 0.1	81 ± 4	6	4.4 ± 0.1	40 ± 7
CHF (substance + NPY)	10	6.4 ± 0.2[b]	111 ± 2	11	6.7 ± 0.2	113 ± 4	10	7.9 ± 0.1[b]	82 ± 4	6	4.5 ± 0.1	44 ± 11
CHF (substance + NPY + BIBP3226)	5	5.8 ± 0.1	116 ± 3	–	–	–	6	7.5 ± 0.2	76 ± 9	–	–	–

Values represent means ± standard error of the mean. *n* refers to the number of rats from which the mesenteric arteries were collected, pEC_{50} refers to the negative logarithm of the molar concentration of agonist inducing a half maximum response, E_{max} refers to the maximum effect on contraction as percent of the potassium-induced contraction.
[a]Substance refers to the contractile agonists. [b]$P < 0.05$ vs. agonist alone (Wilcoxon signed-rank test).

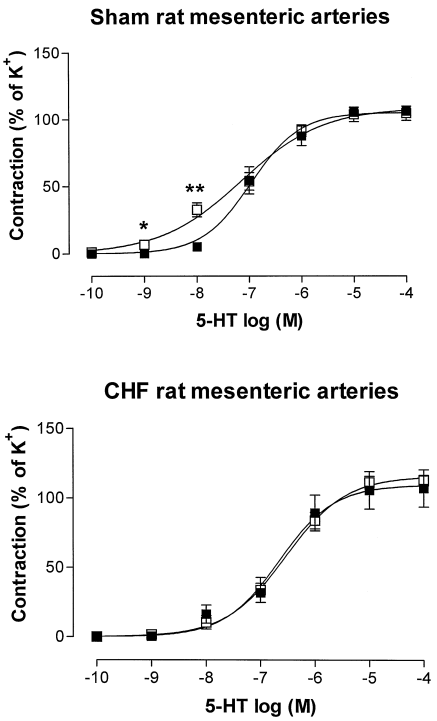

Fig. 3. The contractile response to 5-HT in the absence (■) and presence (□) of neuropeptide Y (10 nM) in sham and congestive heart failure rat mesenteric arteries. The results are expressed as percentages of the K^+-induced contraction, each point represents mean ± standard error of the mean of 9–11 experiments. $* P < 0.05$ (Wilcoxon signed-rank test).

tricle, as confirmed by microscopical evaluation. This was not seen in the sham-operated animals. Evaluation was made according to earlier reports (Pfeffer et al., 1979; Bergdahl et al., 1995). Rats with an infarct size exceeding 30% of the left ventricular circumference were included in the study.

3.2. Potassium-induced contractions

The K^+ (60 mM) buffer solution caused strong contractions in all mesenteric arteries tested. In congestive heart failure rats, the contraction was significantly stronger than in sham-operated rats; 6.8 ± 0.3 mN vs. 5.8 ± 0.3 mN ($p < 0.05$, $n = 40$).

3.3. Effects of neuropeptide Y per se

Neuropeptide Y in concentrations up to 1 μM did not, per se, cause any contraction of the mesenteric arteries neither in congestive heart failure nor in sham animals. Neuropeptide Y, up to 1 μM, failed to induce relaxation in precontracted vessels.

3.4. Effects and potentiation of endothelin effects

Endothelin-1 induced an equally strong concentration-dependent contraction of arteries from congestive heart failure and from sham-operated rats. The congestive heart failure mesenteric arteries were less sensitive to endothelin-1 than those from sham rats; pEC_{50}: 8.2 ± 0.1 and 8.5 ± 0.1, respectively, $P < 0.05$ (Table 1).

Neuropeptide Y and neuropeptide Y-(13–36) (10 nM) potentiated the endothelin-1-induced contraction in arteries from congestive heart failure but not from sham-operated rats. A leftward shift of the concentration–response curve without change of maximum contractile effect was induced (Fig. 1). BIBP3226 (1 μM) failed to inhibit this potentiation (Table 1). FR139317 (1 μM) significantly inhibited the endothelin-1-induced contraction and abolished the potentiation induced by neuropeptide Y (Table 1).

Sarafotoxin 6c in concentrations up to 1 μM, failed to induce contraction in arteries from sham-operated rats. In congestive heart failure 20% of the mesenteric arteries responded to sarafotoxin 6c; E_{max}: $31 \pm 4\%$, pEC_{50}: 8.6 ± 0.4 ($n = 11$). Neuropeptide Y did not potentiate this contraction.

3.5. Potentiation of U46619-induced contraction

U46619 induced an equally strong and potent concentration-dependent contraction of congestive heart failure and sham rat arteries. In congestive heart failure, but not in

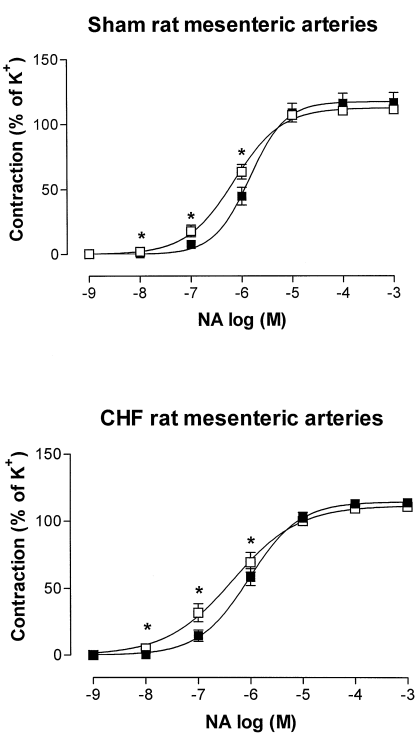

Fig. 4. The contractile response to noradrenaline in the absence (■) and presence of (□) neuropeptide Y (10 nM) in sham and congestive heart failure rat mesenteric arteries. The results are expressed as percentages of the K^+-induced contraction, each point represents mean ± standard error of the mean of 9–11 experiments. $* P < 0.05$ (Wilcoxon signed-rank test).

sham mesenteric arteries, the contraction was significantly potentiated by neuropeptide Y (10 nM) without change in the maximum contractile response (Fig. 2). The potentiation was significantly inhibited by 1 μM BIBP3226 (Table 2).

3.6. Potentiation of 5-HT-induced contraction

5-HT induced an equally strong and potent concentration-dependent contraction of congestive heart failure and sham mesenteric arteries. In sham, but not in congestive heart failure arteries, neuropeptide Y (10 nM) significantly potentiated the 5-HT-induced contraction, causing a leftward shift of the concentration–response curve without change in the maximum contractile response (Fig. 3). The potentiation was significantly inhibited by 1 μM BIBP3226 (Table 2).

3.7. Potentiation of noradrenaline-induced contraction

Noradrenaline induced an equally strong and potent concentration-dependent contraction of congestive heart failure and sham mesenteric arteries. Neuropeptide Y (10 nM) significantly potentiated the noradrenaline-induced contraction, causing a leftward shift of the concentration–response curve without change in the maximum contractile effect, in both congestive heart failure and sham rats (Fig. 4). The potentiation was significantly inhibited by 1 μM BIBP3226 (Table 2).

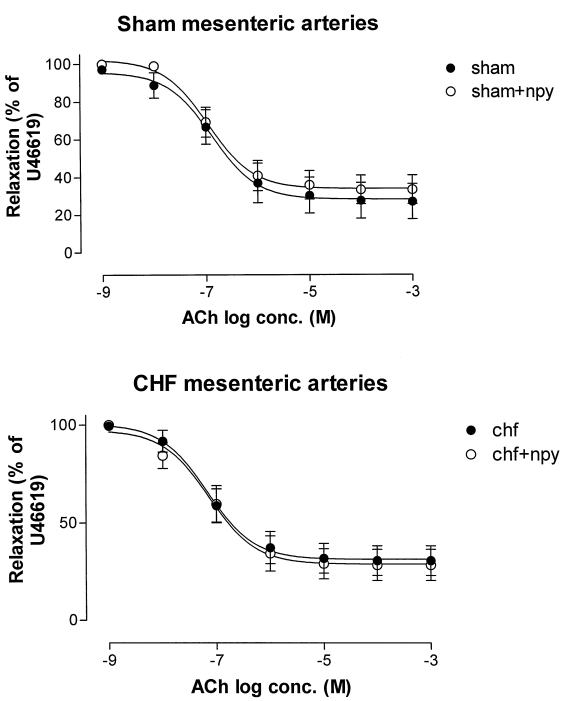

Fig. 5. The relaxant response to acetylcholine in the absence (●) and presence (○) of neuropeptide Y (10 nM) in sham and congestive heart failure rat mesenteric arteries. The result are expressed as percentages of the U46619-induced contraction, each point represents mean ± standard error of the mean of eight experiments.

3.8. Potentiation of ATP-induced contraction

ATP induced an equally strong and potent concentration-dependent contraction of congestive heart failure and sham rat mesenteric arteries. Neuropeptide Y (10 nM) failed to potentiate the contraction in both congestive heart failure and sham rats (Table 2).

3.9. Acetylcholine-induced relaxation

Acetylcholine induced an equally strong and potent concentration-dependent relaxation in precontracted vessels from congestive heart failure and sham rats; pEC_{50} 7.2 ± 0.2 vs. 6.7 ± 0.2 respectively. Neuropeptide Y (10 nM) did not influence the relaxation in congestive heart failure or sham mesenteric arteries (Fig. 5).

4. Discussion

Since the direct effect of neuropeptide Y on isolated peripheral arteries is weak (Grundemar and Högestätt, 1992; Nilsson et al., 1996c) we studied the potentiating effect of neuropeptide Y on various vasoactive agents on mesenteric arteries from congestive heart failure and sham rats. Since elevated levels of noradrenaline have been reported for this model, correlating with changes in adrenergic receptor characteristics (Feng et al., 1996) we assumed that there might be changes in the neuropeptide Y receptor characteristics, particularly since neuropeptide Y is released together with noradrenaline (Grundemar and Håkanson, 1993).

As found in other vascular beds (Bergdahl et al., 1995) the K^+-induced contraction was stronger in congestive heart failure mesenteric arteries. Since neuropeptide Y, ATP and noradrenaline can stimulate the growth of cultured vascular smooth muscle cells, the increased sympathetic activity reported for congestive heart failure may induce hypertrophy or hyperplasia in blood vessels (Blaes and Biossel, 1983; Erlinge et al., 1993, 1994). Thus, the enhanced K^+-induced contraction in congestive heart failure vessels might be the result of an increased number of and/or enlarged vascular smooth muscle cells.

Neuropeptide Y, per se, failed to induce contraction of either congestive heart failure or sham rat mesenteric arteries at concentrations up to 1 μM. Even though neuropeptide Y has been found to be a powerful vasoconstrictor of cerebral (Nilsson et al., 1996a), coronary (Franco-Cereceda, 1989) and subcutaneous arteries (Nilsson et al., 1996b), not all vessels respond to neuropeptide Y (Edvinsson et al., 1984; Ekblad et al., 1984; Grundemar

and Högestätt, 1992). In blood vessels such as omental and mesenteric arteries neuropeptide Y has weak direct contractile effects. Furthermore the direct contractile effect of neuropeptide Y varies between species, vascular beds and vessel diameter, being more prominent in small (resistance) arteries (Uddman et al., 1985).

Neuropeptide Y has been shown to potentiate the effect of various vasoconstricting agents including noradrenaline, histamine, 5-HT and prostaglandin $F_{2\alpha}$ (Andriantsitohaina and Stoclet, 1988; Edvinsson et al., 1984). The mechanism behind the potentiating effect of neuropeptide Y is not well understood but may be related to the mobilisation of both intracellular and extracellular calcium (Wahlestedt et al., 1985; Andriantsitohaina et al., 1993). Further, it has been shown that this mechanism involves a slight depolarisation of the cell membrane (Andriantsitohaina and Stoclet, 1988). A third mechanism that may be involved is the second messenger system since, when a cell line expressing both the neuropeptide Y Y_1 receptor and the α_1-adrenoceptor is stimulated by a combination of α_1 and neuropeptide Y receptor agonists this results in a synergistic increase in the generation of inositol 1,4,5-trisphosphate and potentiation of the α_1-agonist-induced phospholipase A_2 activity even though, alone, neuropeptide Y had no effect (Shine et al., 1994).

Endothelin-1 induced strong concentration-dependent contractions in arteries from both congestive heart failure and sham rats. The contraction was antagonised by FR139317 (a specific endothelin ET_A receptor antagonist), indicating an endothelin ET_A receptor-mediated contraction (data not shown). As seen before in coronary arteries from dogs with experimental heart failure (Cannan et al., 1996), we obtained an attenuated contractile response of endothelin-1 in the congestive heart failure rat arteries. This is consistent with a report by Fu et al. (1993) of a decreased density of endothelin receptor binding in mesenteric arteries from congestive heart failure rats. Neuropeptide Y potentiated the endothelin-1-induced contraction in congestive heart failure but not in sham rats. This is in agreement with observations in vivo of potentiation of the neuropeptide Y effect on the pressor response to endothelin-1 in rats with congestive heart failure (Sun et al., 1995).

Notably, the neuropeptide Y Y_1 receptor antagonist, BIBP3226, failed to antagonise the potentiation of the endothelin-1 contractions. This effect seems therefore not to be mediated by the neuropeptide Y Y_1 receptor, which is the receptor usually thought to be involved in the potentiation (Bergdahl et al., 1996). The potentiating effect of the neuropeptide Y Y_2 agonist, neuropeptide Y-(13–36) is further evidence that another receptor involved, presumably the neuropeptide Y Y_2 receptor.

In 20% of the congestive heart failure rats, but in none of the sham rats, sarafotoxin 6c induced a $31 \pm 4\%$ contraction, suggesting an increased number of contractile endothelin ET_B receptors. Our findings concur with those of Cannan et al. (1996) who demonstrated an enhanced coronary vasoconstriction in response to sarafotoxin 6c in dogs with experimental heart failure. Neuropeptide Y failed to potentiate the sarafotoxin 6c-induced contraction. Thus there are both altered endothelin ET_A and endothelin ET_B receptor responses, together with changes in the neuropeptide Y receptor subtype that mediates the potentiation in arteries from rats with congestive heart failure.

Increased formation of the cyclooxygenase-derived vasoconstricting factors, thromboxane A2 and prostaglandin $F_{2\alpha,}$ have been reported in humans with congestive heart failure (Katz et al., 1993; Castellani et al., 1997). Forster et al. (1992) demonstrated an enhanced response to prostaglandin $F_{2\alpha}$ in dogs with heart failure, while there was no difference in U46619-induced contractions of arteries from sham-operated and congestive heart failure rats in our study. However, neuropeptide Y potentiated the U46619-mediated contraction in congestive heart failure, but not in sham mesenteric arteries. U46619 has been shown to increase the Ca^{2+} sensitivity of the vascular contractile elements (Himpens et al., 1990; Crichton et al., 1993) and K^+-stimulated contractility was found to be enhanced in our experiments. Thus, the neuropeptide Y induced potentiation of the U46619 effect could be due to a synergistic effect in heart failure vessels, these vessels being more sensitive and having an enhanced contractile capacity in response to Ca^{2+} influx. The potentiation was abolished by BIBP3226, suggesting a neuropeptide Y Y_1-receptor mediated effect.

The 5-HT-mediated contraction in mesenteric arteries did not differ in either potency or in maximum effect between arteries from congestive heart failure and sham-operated rats. Similar observations have been reported (Angus et al., 1993). Neuropeptide Y potentiated the 5-HT-induced contraction in sham rats, an effect seen in the rat resistance vasculature as well (Andriantsitohaina and Stoclet, 1988). The potentiation was inhibited by BIBP3226. In congestive heart failure animals, however, neuropeptide Y did not induce any potentiation. This indicates a loss of effect of the neuropeptide Y ($-Y_1$) receptor, being downregulated or otherwise affected in congestive heart failure.

It is well known that patients with congestive heart failure have elevated plasma levels of noradrenaline (Edvinsson et al., 1990). In the congestive heart failure rat arteries we observed elevated levels of noradrenaline together with a decreased responsiveness of vascular postjunctional α_2-adrenoceptors (Feng et al., 1996) while the heart failure dog has an increased sensitivity to α_1-adrenoceptor stimulation (Forster and Armstrong, 1990). In our study, there were no differences in noradrenaline-induced contractions between the two groups. Further there was no difference in the potentiating effect of neuropeptide Y in congestive heart failure and sham rats. Since neuropeptide Y has been shown to restore the pressor response to noradrenaline in noradrenaline-desensitised rats (Wahlestedt et al., 1990) and since the synergistic effect of

α_1-adrenoceptors and neuropeptide Y is very strong (Shine et al., 1994) a presumed attenuation or downregulation of the α-adrenoceptor- or neuropeptide Y receptor-mediated response might have been overcome by these mechanisms. It is concluded that more work is needed in this particular area of research. The potentiation of the noradrenaline-induced contraction was inhibited by BIBP3226, indicating a neuropeptide Y Y_1 receptor-mediated effect.

The ATP-mediated contraction did not differ as to neither potency or maximum contractile response between congestive heart failure and sham rat mesenteric arteries. Furthermore, neuropeptide Y failed to induce potentiation of ATP responses in the vessels studied, while this has been shown for the guinea pig saphenous vein (Cheung, 1991). The plasma level of adenosine has been reported to be elevated in humans with congestive heart failure, reflecting an augmented level of ATP (Funaya et al., 1997). The lack of effect of neuropeptide Y is surprising since the potentiating effect has been shown to be, in part, dependent on the purinoceptors (Cheung, 1991). However, the potentiating effect is vessel-dependent (Edvinsson et al., 1984) and might even be species-dependent, thus partly explaining the results obtained.

Finally, we investigated the relaxation induced by acetylcholine. We observed no difference in the acetylcholine mediated relaxation in mesenteric arteries from congestive heart failure rats and those from sham rats. This is in agreement with results from dogs with heart failure (Forster et al., 1992) but not with results reported by Angus et al. (1993) who found a decreased response to acetylcholine in humans with congestive heart failure. The lack of inhibitory effect of neuropeptide Y on acetylcholine-induced relaxation, an effect reported by others (Grundemar and Högestätt, 1992) and by our own group (Edvinsson and Adamsson, 1992; Nilsson et al., 1996c) might reflect a variation of the receptor population depending on the vascular region and species studied.

In summary and conclusion, the most important findings from this study were: (1) decreased vascular response to endothelin-1, increased vascular response to sarafotoxin 6c and a neuropeptide Y-induced potentiation of endothelin-1 contractions. This effect was not blocked by BIBP3226 in arteries from congestive heart failure. (2) A neuropeptide Y Y_1 receptor-mediated potentiation of the U46619-induced contraction, antagonised by BIBP3226 in arteries from congestive heart failure but not sham rats, (3) a loss of the potentiation by neuropeptide Y of 5-HT-induced contractions in arteries from congestive heart failure compared to those from control rats. Since plasma levels of endothelin-1 and arachidonic acid-derived metabolites (prostaglandin $F_{2\alpha}$ and thromboxane A_2), which contribute to increased vascular resistance are elevated in patients with congestive heart failure, the potentiating effect of neuropeptide Y on endothelin-1- and prostaglandin $F_{2\alpha}$-induced contractions may further increase the vascular tone in congestive heart failure, especially since neuropeptide Y plasma concentrations are elevated in congestive heart failure.

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