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European Journal of Pharmacology 539 (2006) 184–191
www.elsevier.com/locate/ejphar

Enhanced neuropeptide Y immunoreactivity and vasoconstriction in mesenteric small arteries from the early non-obese diabetic mouse

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Received 16 November 2005; received in revised form 27 March 2006; accepted 31 March 2006
Available online 7 April 2006

Abstract

The present study investigated whether sympathetic neurotransmission is altered at an early stage of diabetes in mesenteric small arteries isolated from female non-obese diabetic (NOD) and control animals without diabetes from the same mouse strain. The NOD diabetic mice had increased plasma glucose and hypertension. Confocal microscopy showed distribution of nerve terminals was similar, but immunoreaction intensity for neuropeptide Y (NPY) and tyrosine hydroxylase was higher in small arteries from NOD diabetic compared with NOD control mice. In the presence of prazosin and activated with vasopressin, electrical field stimulation evoked contractions which were more pronounced in mesenteric arteries from NOD diabetic versus NOD control mice and inhibited by the NPY Y_1 receptor antagonist, BIBP 3226. NPY concentration–response curves were leftward shifted in arteries from NOD diabetic versus NOD control both in arteries with and without endothelium, but not in the presence of the BIBP 3226. The present findings suggest that enhanced NPY content and vasoconstriction to NPY by activation of NPY Y_1 receptors in arteries from diabetic mice may contribute to the enhanced sympathetic nerve activity and vascular resistance in female non-obese early diabetic animals.

Keywords: Mesenteric artery; Neuropeptide Y; Electrical field stimulation; Noradrenaline; Acetylcholine; BIBP 3226; (Non-obese diabetic-mice)

1. Introduction

Diabetes is associated with an increased incidence of macro- and microvascular disease (Garcia et al., 1974; Colwell et al., 1979). In addition to hypertension, sympathetic neuropathy may play a role for development of cardiovascular complications in diabetic patients. In the streptozocin-induced diabetic rats which represent an animal model for type-1 diabetes, increased plasma levels and cardiac turnover of noradrenaline were found in early diabetes (Akiyama et al., 1989; Ganguly et al., 1987; Kuncova et al., 2005), while responses to sympathetic nerve stimulation and noradrenaline have been reported as decreased (Ralevic et al., 1995; Takiguchi et al., 1988; Longhurst and Head, 1985; Andersson et al., 1992), or increased (Macleod, 1985; Sjogren and Edvinsson, 1988; Taylor et al., 1992; Savage et al., 1995; Setty et al., 2004) in arteries from diabetic animals.

Apart from noradrenergic innervation, the vasculature receives also peptidergic innervation by neuropeptide Y (NPY)-containing nerve fibres. In diabetic patients sympathetic ganglia and NPY containing axonal terminals have been shown to be dystrophic (Levy et al., 1989; Schmidt et al., 2003). These alterations may cause diminished NPY release and lower circulating NPY in patients with type 1 diabetes (Sundkvist et al., 1992; Wocial et al., 1995). However, in type 2 diabetes and patients with diabetic nephropathy plasma immunoreactive neuropeptide Y concentrations are elevated (Satoh et al., 1999; Milewicz et al., 2000), and central NPY levels are increased in streptozocin-induced diabetic rats (White et al., 1990; Chavez et al., 1998). While cardiac NPY levels in streptozocin-induced diabetic rats decreased later, they were unaltered during the first 9 months (Kuncova et al., 2005).

Abbreviations: BIBP 3226, (R)-N2-(diphenylacetyl)-*N*-8(4-hydroxyphenyl)-methyl]-argininеamide; NPY, neuropeptide Y; EFS, electrical field stimulation; NOD, non-obese diabetic mouse strain; NO, nitric oxide; L-NAME, N^{ω}-nitro-L-arginine methyl ester.

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doi:10.1016/j.ejphar.2006.03.080

NPY levels were also unaltered in the gut of non-obese diabetic mice (Spangeus et al., 2000), but increased in the pancreas of the streptozocin-induced diabetic rat (Adeghate, 2002). In hypertensive patients NPY levels are elevated (Erlinge et al., 1992; Wocial et al., 1995), and in spontaneously hypertensive rats NPY content appears increased in nerves of the cerebral and mesenteric vasculature (Kawamura et al., 1989; Gradin et al., 2003). Therefore, both sympathetic neuropathy and hypertension can contribute to altered responsiveness to sympathetic neurotransmitters in diabetic patients.

There are five NPY receptor subtypes which have been identified by molecular cloning and are designated Y_1, Y_2, Y_4, Y_5 and Y_6 (Michel et al., 1998). NPY-induced vasoconstriction and elevation of mean arterial pressure occur predominantly via postjunctional Y_1 receptors in blood vessels (Michel et al., 1998; Edvinsson et al., 1994; Bischoff et al., 1997). In arteries from hypertensive animals increased responsiveness of NPY Y_1 and Y_2 receptors has been described (Mezzano et al., 1998; Aguirre et al., 1990; Gradin et al., 2003). NPY contraction was attenuated in arteries from patients with type 2 diabetes (Lind et al., 1995), alloxan-induced diabetic rabbits (Andersson et al., 1992), and streptozocin-induced diabetic rats (Hu and Dunbar, 1997). NPY Y_2 receptors are involved in the retinal neovascularization in diabetic retinopathy in mice (Koulu et al., 2004), hence pointing to an altered responsiveness of Y_1 and Y_2 receptors in diabetic animals.

NPY-induced contraction is greater in tail arteries from female compared to male rats, and gonodal hormones appear to modulate several components involved in NPY neurotransmission (Glenn et al., 1997). Moreover, NPY-induced diuresis and natriuresis were similar in male and female normotensive rats, but while these responses were desensitized in male they were augmented in female rats (Bischoff and Michel, 2000). Therefore, the hypothesis of the present study was that sympathetic neurotransmission mediated by NPY is altered at an early stage of diabetes in female diabetic animals. For this purpose mesenteric small arteries were isolated from female non-obese diabetic and control animals without diabetes from the same mouse strain, and immunohistochemistry and functional studies were performed.

2. Materials and methods

2.1. Animal and tissue preparation

All the procedures performed on the animals in the present study were in accordance with Swedish animal law and regulations. Experiments were performed in 10-week old female mice of the non-obese diabetic (NOD) mice, a strain that spontaneously develops insulin-dependent diabetes mellitus (Møllegaard Breeding Center, Skensved, Denmark) weighing 18–30 g. The mice were kept on standard chow and had free access to water, each cage containing 2–5 animals. The mice were allowed to adjust to the new environment for a week before the start of the experiments. The cumulative incidence of overt diabetes among female animals of this strain is >80% in 25 weeks. Sibs of NOD mice which were not diabetic at the time of examination represent the control population (Schmidt et al., 2003). Both NOD control and NOD diabetic 10-week old mice were observed for 2 weeks. Blood for blood-glucose measurements were taken from the tail vein weekly by means of a lancet under isofluorane anaesthesia and measured by the use of a glucose assay (Infinity Glucose Reagent, Sigma Diagnostics, Sigma-Aldrich Company Ltd, Dorset, UK). The tail-cuff method was used for non-invasive measurements of systolic blood pressure, where measurements were repeated for at least 8 times on 2 consecutive days.

At the day of the experiment anaesthesia was induced and continued by inhalation of isofluorane (2–3% V/V, isofluorane, Pharmacia and Upjohn, Stockholm, Sweden) mixed with air (1 L/min) in an isofluorane vaporizer (Ohmeda Isotec 5, Simtec engineering, Askim, Sweden). The body temperature of the mouse was kept at 37 °C during the preparations by means of a thermostatically controlled heating pad and a temperature-sensitive lamp connected to a rectal probe. Control and diabetic mice were anaesthetized by isofluorane and the mesenteric arteries supplying the proximal jejunum were carefully freed from surrounding tissue. Segments of 2 mm length were threaded onto two stainless wires and suspended in 5 mL microvascular myograph baths (Danish Myotechnology, Aarhus, Denmark). The tissue baths were temperature controlled (37 °C) and contained physiological saline solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, glucose 5.5, KCl 4.7, $CaCl_2$ 2.5, KH_2PO_4 1.18, $MgSO_4$ 1.17, and ethylenediaminetetraacetic acid 0.026. High potassium salt solution was similar to physiological salt solution except that NaCl was exchanged with KCl on an equimolar basis. Solutions were equilibrated with 5% CO_2 in O_2 to maintain a pH of 7.4. The vessels were allowed to stabilize for 60 min. The relation between resting wall tension and internal circumference was determined, and from this the internal circumference L_{100} corresponding to a transmural pressure of 100 mm Hg for a relaxed vessel in situ was calculated. The vessels were set to the internal circumference L_1, given by $L_1 = 0.9L_{100}$.

2.2. Immunohistochemistry for NPY and tyrosine hydroxylase

The vessels were incubated with calcium-free sodium phosphate-buffered saline followed by immersion in 4% paraformaldehyde. After 20 h the vessels were placed in chilled PBS until analysis. Indirect immunofluorescence incubations were carried out using the following primary antibodies: rabbit anti-neuropeptide Y (NPY), produced against synthetic NPY (Sigma), dilution 1:4000, rabbit anti-tyrosine hydroxylase, produced against tyrosine hydroxylase isolated from human pheochromocytomas, dilution 1:800. Afterwards the second antibody was used, which was donkey anti-rabbit conjugated with fluorescein isothiocyanate conjugate (Jackson, ImmunoLab, Grove, USA).

The quantitative analysis of immunoreactivity for NPY and tyrosine hydroxylase was carried out on single confocal image using Pixel Anatomy software as described previously (Gradin et al., 2003). Briefly, as a first step, background fluorescence was estimated by analysing the distribution of pixel intensities

in the image areas that did not contain any immunolabelled objects (the background threshold). The background was subtracted by setting the baseline of pixel intensities to the background value. In the next step, an arbitrary outlined polygon was chosen, which covered the vessel-occupied and imaged area, for quantification of immunoreactivity for NPY and tyrosine hydroxylase. In the polygon area chosen for analysing the relative area (%) of immunolabelled pixels with an intensity value above the background was calculated. Images were then processed using Adobe Photoshop program.

2.3. Recording of mechanical activity

The contractile ability of the vessels was tested by stimulating with high potassium saline solution (124 mM) until reproducible responses were obtained, i.e. when the active developed contraction was within 10% of the previous contraction to high potassium saline solution. In almost all experiments this was reached with a third stimulation to high potassium saline solution.

Electrical field stimulation (EFS) was performed with platinum electrodes (Danish Myotechnology, Aarhus, Denmark), measuring 2×2 mm, which were secured in plastic mounting heads on either side of the artery, approximately 1 mm from the vessel wall. The electrodes were connected to an electrical stimulator (Danish Myotechnology, Aarhus, Denmark) with constant current output adjusted to 35 mA as previously described (Gradin et al., 2003). The arteries were treated with prazosin (3 μM) and contracted with vasopressin, and EFS (16 Hz) was applied as 90 s trains.

The effect of L-NAME on resting tension and acetylcholine relaxation was examined. A first concentration–response curve for acetylcholine (10^{-9}–10^{-5} M) was constructed in noradrenaline (10^{-8}–10^{-5} M) precontracted arteries, and a second after incubation with the NO synthase inhibitor, N^{W}-nitro-L-arginine methyl ester (L-NAME, 1 μM) for 30 min.

The effect on concentration–response curves for NPY of the Y_1 receptor-selective antagonist, BIBP 3226, was evaluated. Arteries with endothelium were treated with BIBP 3226 (0.3 μM) and contacted with vasopressin (0.5–0.8 U L^{-1}) and when the contraction was stable, increasing concentrations of NPY (10^{-12}–10^{-7} M) were added. To examine whether these responses were endothelium-dependent, the endothelial cells were removed by insertion of a wire into the lumen of the artery and gently rubbing the internal surface back and forth for 5 min. Abolished acetylcholine relaxation was taken as for successful removal of the endothelium.

2.4. Drugs and solutions

The following drugs were used: neuropeptide Y (NPY, human) noradrenaline, acetylcholine, vasopressin, prazosin, L-NAME (Sigma, USA), (R)-N^2-(diphenylacetyl)-*N*-[4-hydroxyphenyl)-methyl]-arginineamide (BIBP 3226) was a gift from Dr. Stephan Mueller (Dr. Karl Thomae GMBH, Germany). Drugs were dissolved in distilled water, except for BIBP 3226 which were dissolved in dimethyl sulphoxide and further diluted in water. Stock solutions were prepared and stored at −20 °C and further fresh dilutions were prepared daily. The resulting concentration of dimethyl sulphoxide (0.03%) in the organ bath had no effect on the preparations.

2.5. Analysis of data and statistics

For quantification of nerves immunoreactive for tyrosine hydroxylase and NPY were statistically processed with Statview software. Images were then processed using Adobe Photoshop program.

Mechanical responses of vessels were measured as force and expressed as active wall tension (ΔT) which is the increase in force above baseline (ΔF), divided by twice the segment length. Sensitivity to the agonists is given as pD_2 values, where $pD_2 = -\log EC_{50}$ (*M*), EC_{50} being the concentration (*M*) of agonist required to give half-maximal response. The results are expressed as mean±S.E.M. and *n* represents the number of arteries (one per animal). Statistical differences between groups were tested by the use of two-way analysis of variance (2-way ANOVA), Student's paired, or unpaired *t*-test when appropriate. Probability levels below 5% were considered statistically significant.

3. Results

3.1. Animal data

The body weight was unchanged in the different treatment groups compared to control. The systolic tail blood pressure was 135±0.5 mm Hg (n=5) in NOD control mice and markedly increased in 10 week NOD diabetic mice (165±4.0 mm Hg,

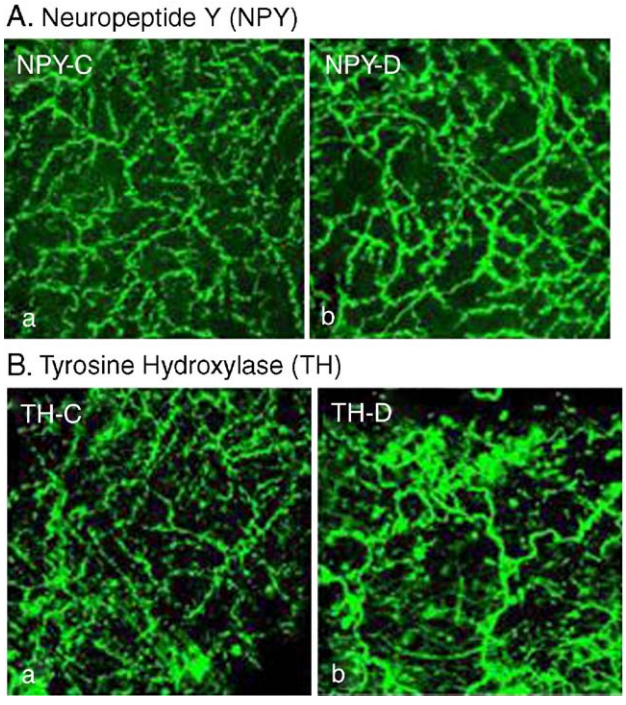

Fig. 1. Distribution of nerves immunoreactive for NPY and tyrosine hydroxylase in mesenteric small arteries from NOD control and diabetic mice. The distribution and pattern of immunoreactivity for (A) NPY and (B) tyrosine hydroxylase (TH) is similar between NOD control (a) and NOD diabetic (b) mice in whole mount preparations.

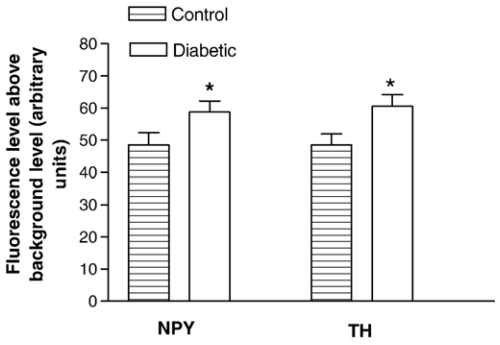

Fig. 2. Quantitative analysis showing that the intensity of the immunoreaction for NPY and tyrosine hydroxylase (TH) was higher in arteries from the NOD diabetic mice than that from NOD control mice. The fluorescence level above background level (see Materials and methods) is shown on the *Y*-axis. Each point and bar represents mean±S.E.M. of tissue from 5 animals. Differences were evaluated by Student's *t*-test. **P*<0.01.

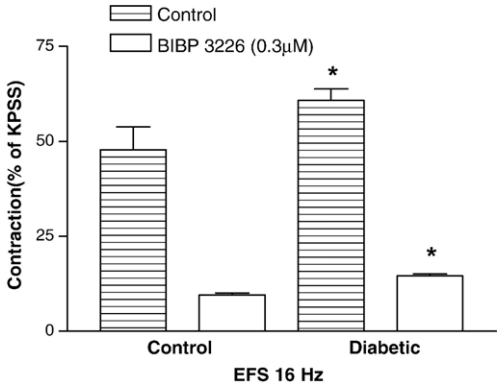

Fig. 3. Average vasoconstriction responses to EFS (16 Hz for 90 s) in prazosin (3 μM)-treated and vasopressin (0.5 U L^{-1})-activated arteries from control and diabetic NOD mice. Each point and bar represents the mean±S.E.M. of arteries from 5 animals. Significantly different responses evaluated by *t*-test: **P*<0.05 versus control.

P<0.01, *n*=5). The glucose levels were significantly increased in NOD diabetic (44±8 mM, *n*=5) compared to NOD control mice (9.0±0.7 mM, *n*=5).

3.2. Immunofluorescence for NPY and tyrosine hydroxylase

The lumen diameter of mesenteric small arteries isolated from NOD control mice was 213±8 μm and was markedly smaller (172±3.5 μm, *P*<0.01, *n*=5) in arteries from NOD diabetic mice. Immunoreactive fibres for tyrosine hydroxylase and NPY were localized in the adventitia of mesenteric small arteries. The distribution pattern of immunoreactive fibres was similar in whole mount arterial preparations isolated from NOD control and NOD diabetic mice (Fig. 1). However, the NPY and tyrosine hydroxylase immunoreaction intensity was more pronounced in arteries from NOD diabetic compared to NOD control mice, and quantitative analysis revealed intensity for NPY and tyrosine hydroxylase was significantly higher in mesenteric arteries from NOD diabetic compared with NOD control animals (Fig. 2).

Table 1
Contractile responses induced by 124 mM K^+ rich solution, an inhibitor of NO synthase, L-NAME (1 μM), noradrenaline, and neuropeptide Y (NPY) in arteries with (+E) and without (−E) from NOD control and NOD diabetic mice

	NOD control		NOD diabetes	
Parameter	pD_2	ΔT (N m^{-1})	pD_2	ΔT (N m^{-1})
124 mM K^+	–	2.10±0.10	–	2.10±0.10
L-NAME	–	0.18±0.03	–	0.40±0.04[a]
NA	7.36±0.08	1.66±0.08	7.64±0.02[a]	1.92±0.05[a]
NPY (+E)	7.45±0.01	1.09±0.06	8.00±0.01[a]	1.40±0.08[a]
NPY (−E)	8.07±0.01[b]	1.28±0.04[b]	8.52±0.05[a,b]	1.57±0.0[a,b]
NPY+BIBP (+E)	7.45±0.05	0.94±0.05[c]	7.12±0.04[a,c]	0.92±0.09[c]
NPY+BIBP (−E)	7.71±0.10[b,c]	1.15±0.05[b]	7.61±0.30[b,c]	1.28±0.03[b,c]

Curves for NPY were obtained in the absence and the presence of BIBP 3226 (300 nM).
Results are means±S.E.M. of arteries from 6 animals for each parameter. Differences in responses evaluated by Student's *t*-test or two-way analysis of variance: [a]*P*<0.05 versus NOD-C, [b]*P*<0.05 versus vessel with endothelium, [c]*P*<0.05 versus response constructed in the absence of BIBP 3226.

3.3. Functional studies

High extracellular potassium saline solution (124 mM) contracted first order mesenteric arteries from NOD control and NOD diabetic mice to similar level, while contractions induced by an inhibitor of NO synthase, L-NAME (1 μM) were doubled in arteries from NOD control versus NOD diabetic animals (Table 1). In mesenteric arteries incubated with prazosin and precontracted with vasopressin, EFS (16 Hz, 90 s trains) evoked contractions (Fig. 3), which were abolished after guanethidine treatment (*n*=3, results not shown). Expressed as a percentage of contractions evoked by high potassium saline solution (124 mM), the contractions induced by EFS were significantly enhanced in arteries from NOD diabetic compared to arteries from NOD control mice. BIBP 3226 inhibited EFS-evoked contraction in arteries from NOD diabetic and NOD control mice (Fig. 3).

Noradrenaline evoked concentration-dependent contractions which were shifted leftward in mesenteric arteries from diabetic

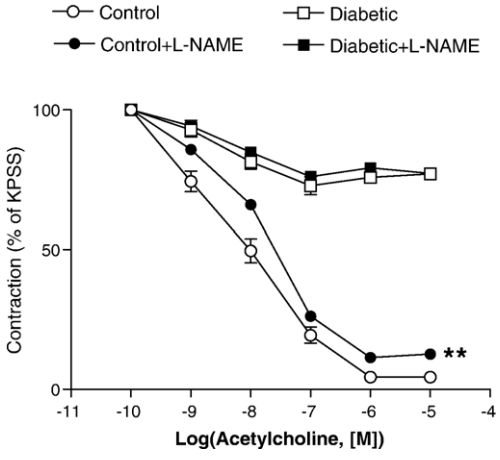

Fig. 4. Relaxation curves for acetylcholine in the absence and presence of an inhibitor of NO synthase, L-NAME (1 μM), in mesenteric arteries from control and diabetic NOD mice. Responses are expressed as percentage of the relaxation induced by noradrenaline. Each point and bar represents mean±S.E.M. of arteries from 5 animals. Significantly different responses evaluated by *t*-test: ***P*<0.01 versus control.

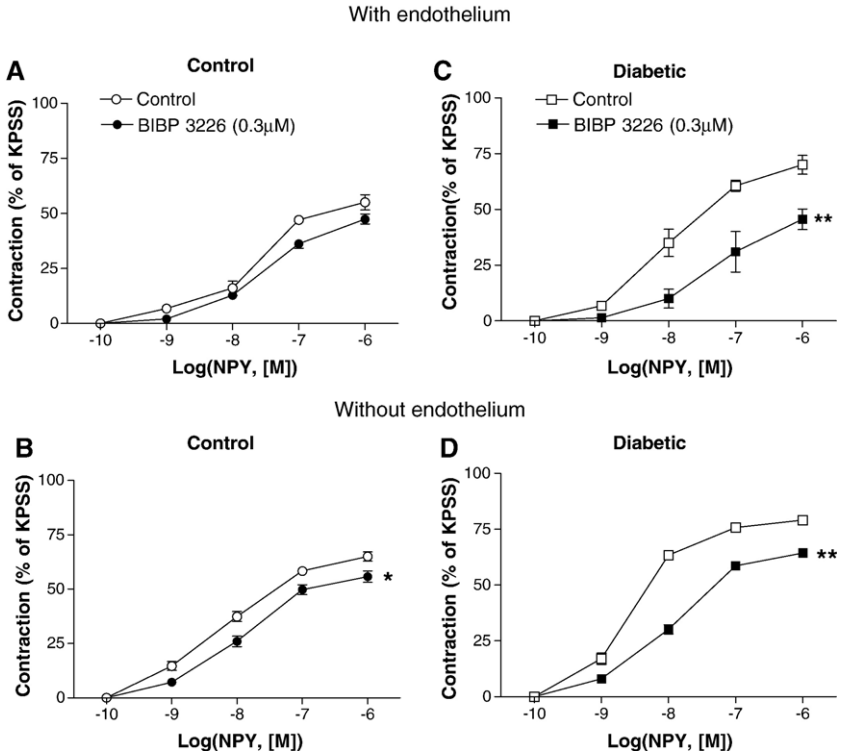

Fig. 5. Concentration–response curves for neuropeptide Y (NPY) obtained in vasopressin (0.5 U L^{-1}) preactivated mesenteric small arteries from (A, B) control and (C, D) female non-obese diabetic mice. Concentration–response curves were obtained in arteries with and without endothelium and the absence and the presence of the Y_1 receptor antagonist BIBP 3226 (0.3 μM). Responses are expressed as percentage of the contraction induced by 124 mM high potassium saline solution (KPSS). Each point and bar represents mean±S.E.M. of arteries from 5 animals. Differences in responses evaluated by two-way analysis of variance: *$P<0.05$, **$P<0.01$, arteries with versus arteries without BIBP 3226.

compared to control arteries; also the maximal contraction evoked by noradrenaline was increased in arteries from NOD diabetic versus NOD control animals (Table 1). In noradrenaline-contracted arteries from NOD control mice, acetylcholine evoked concentration-dependent relaxations, which were markedly inhibited in the presence of L-NAME. In the absence and the presence of L-NAME, relaxation evoked by acetylcholine was less in arteries from NOD diabetic versus NOD control mice (Fig. 4).

In arteries incubated with the α_1-adrenoceptor antagonist prazosin (10 μM), vasopressin (0.5 U L^{-1}) induced a rapid contraction which peaked after 3–4 min and reached 2.1±0.2 N m^{-1} ($n=5$) and 2.4±0.1 N m^{-1} ($n=5$), respectively, in arteries from NOD control and NOD diabetic mice. Within 10–15 min the vasopressin-evoked contraction stabilized at a lower level and was maintained for up to 1 h. In vasopressin-activated mesenteric arteries, NPY evoked concentration-dependent contractions which were more pronounced in arteries from NOD diabetic compared to NOD control animals (Fig. 5). Thus, NPY concentration–response curves were leftward shifted and maximum response increased in arteries from NOD diabetic versus NOD control mice (Fig. 5). The leftward shift in concentration–response curves for NPY was observed both in arteries with and without endothelium, but not in the presence of the NPY Y_1 receptor antagonist, BIBP 3226 (0.3 μM) (Table 1, Fig. 5).

4. Discussion

The main finding of the present study is that NPY neurotransmission seems altered in arteries from female non-obese diabetic animals. This is supported by the observations of enhanced immunoreactivity for NPY and enhanced contractions induced by EFS and exogenously added NPY. Alterations in Y_1 receptors in arteries from diabetic mice may contribute to the enhanced vasoconstriction.

The non-obese diabetic mice spontaneously develop diabetes due to autoimmune attack on its pancreatic islets, and hence they represent an animal model of type 1 diabetes (Akashi et al., 1997; Schmidt et al., 2003). They have a shortened lifespan of 5–8 weeks after the onset of diabetes unless treated with insulin. However in addition to being hyperglycemic, the diabetic animals in the present study were also hypertensive. The NOD mice were developed from the cataract-prone ICR mouse and measurements in anaesthetized animals did not show increased blood pressure comparing NOD mice and outbreed ICR mice (Carlsson et al., 1998). However, blood pressure in NOD mice increases with age (Carlsson et al., 1998), and in the present study comparison with non-diabetic controls of the same mouse strain revealed blood pressure in conscious animals was markedly increased. Therefore, in the present study both diabetes and hypertension have effect on sympathetic neurotransmission in NOD mice.

4.1. Altered neurotransmission in NOD mice

In the present study immunoreactivity for NPY and tyrosine hydroxylase was increased in nerve fibres in mesenteric arteries from NOD mice. The distribution of nerve fibres did not appear different in arteries from diabetic versus control animals. These findings apparently contrast to description of dystrophic sympathetic ganglia and NPY containing axonal terminals in tissue from diabetic patients (Schmidt et al., 2003), and lower levels of circulating NPY in patients with type 1 diabetes (Sundkvist et al., 1992; Wocial et al., 1995). However, we and others have previously found increased nerve content of immunoreactivity for NPY (Kawamura et al., 1989; Gradin et al., 2003), tyrosine hydroxylase (Cassis et al., 1985; Scott and Pang, 1983), or noradrenaline (Donohue et al., 1988) in vascular segments from hypertensive rats. Increased muscular sympathetic nerve activity was found in diabetic patients who also suffered from hypertension (Huggert et al., 2003), and pressor and sympathetic nerve response to electrical stimulation of ventromedial hypothalamus was also enhanced in deoxycorticosterone acetate salt hypertension in streptozotocin diabetic rats (Sasaki and Bunag, 1982). Therefore, it is likely that the enhanced immunoreactivity for NPY and tyrosine hydroxylase in mesenteric arteries may be ascribed to the presence of hypertension in the NOD mice, although we cannot exclude the stage of diabetes also influences the innervation in the animals, since NPY immunoreactivity seems to be depleted in mesenteric arteries from older NOD mice (Gradin, unpublished).

In the presence of prazosin to inhibit the adrenergic neurotransmission, intense EFS evoked neurogenic contractions which were enhanced in NOD diabetic versus NOD control mice. The non-peptide receptor antagonist for NPY Y_1 receptors, BIBP 3226, has made it possible to address the functional role of NPY in sympathetic vasoconstriction (Michel et al., 1998), and the antagonist caused a pronounced reduction in EFS-evoked vasoconstriction in arteries from both NOD control and NOD diabetic mice. These findings confirm earlier studies suggesting that Y_1 receptors are involved in neurogenic contractions of mesenteric small arteries and is probably the main NPY population involved in the enhanced EFS contraction in arteries from NOD mice. In addition to the Y_1 receptor, it cannot be excluded that other NPY receptor subtypes are also involved, since the residual EFS contraction persisting in the presence of BIBP 3226 was different in arteries from NOD diabetic versus NOD control mice.

4.2. Increased vasoconstriction evoked by NPY

In the present study concentration–response curves for noradrenaline and NPY were leftward shifted and the maximal responses were increased (Table 1). In arteries from streptozotocin diabetic rats increased responses to agonists have also been ascribed and were addressed to increased calcium influx (White and Carrier, 1990), increased formation of phosphoinositides (Abebe and Macleod, 1992; Mueed et al., 2005), and changed calcium sensitivity (Chow et al., 2001). In contrast to the other vasoconstrictors, contraction evoked by increasing extracellular K^+ was not enhanced in arteries from NOD diabetic versus NOD control animals in the present study suggesting that the enhanced contractility can be ascribed to altered smooth muscle signal transduction pathways rather than to a general increase in calcium influx. Moreover, lumen diameter of the mesenteric arteries from the NOD diabetic was small compared to NOD control mice, and therefore the increased contraction of the arteries is probably also in large part due to structural remodelling of the arterial wall.

Altered endothelial function can also lead to enhanced agonist-induced contraction in arteries. Dysfunction of the vascular endothelium is regarded as an important factor in the pathogenesis of diabetic micro- and macro-angiopathy, and blunted acetylcholine relaxation was described in resistance arteries from subjects with type 1 diabetes (Mc Nally et al., 1994), and in arteries from stretozotocin-induced diabetic rats (Taylor et al., 1992, 1994). In the present study, the endothelium-dependent vasodilator acetylcholine induced also less maximal relaxation in arteries from diabetic versus control mice. NO has previously been shown to inhibit the release of endothelium-derived hyperpolarizing factor (Bauersachs et al., 1996). L-NAME contraction was enhanced in arteries from NOD diabetic versus NOD control mice in the present study, and these results suggest increased basal formation of NO may contribute to the reduced acetylcholine relaxation in arteries from diabetic animals in the present study.

The endothelium appears to have both NPY Y_1 and Y_2 receptor subtypes (Zukowska-Grojec et al., 1998), and recent studies have described that endothelial NPY receptors mediate vasodilatation in renal (Torffvit and Edvinsson, 1997), cerebral (You et al., 2001), and mesenteric arteries (Gradin et al., 2003). In the present study endothelial cell removal induced a leftward shift of concentration–response curves for NPY in arteries from both NOD diabetic and control mice suggesting basal release of endothelium-derived vasodilators counteract the contractile effect of NPY in mesenteric arteries. However, the leftward shift in concentration–response curves for NPY was similar in arteries from NOD diabetic and control mice. Therefore, endothelial cell dysfunction does not appear to contribute to the enhanced NPY-evoked contraction in mesenteric arteries from NOD mice.

The increased maximal contraction and leftward shift of concentration–response curves for NPY persisted in arteries without endothelium from diabetic animals. However, in the presence of the NPY Y_1 receptor antagonist, BIBP 3226, concentration–response curves for NPY were similar in arteries from NOD mice. These findings suggest coupling of the Y_1 receptor is altered in the vascular smooth muscle from NOD mice. In rat mesenteric arteries Y_1 was found to cause contraction by depolarization, calcium influx through both nifedipine-sensitive and insensitive calcium channels, and inhibition of cyclic AMP (Andriantsitohaina et al., 1993; Prieto et al., 2000). Therefore, in addition to the enhanced immunoreactivity for NPY in the vessel wall altered coupling of the NPY Y_1 receptor in the vascular smooth muscle is a likely explanation for the enhanced neurogenic contractions observed in mesenteric arteries from NOD mice, although further studies should address

which intracellular mechanisms coupled to the Y_1 receptors are altered in mesenteric vascular smooth muscle from NOD mice.

The present investigation suggests that enhanced NPY content and vasoconstriction to NPY by activation of NPY Y_1 receptors in arteries from diabetic mice may contribute to enhanced sympathetic nerve activity in female non-obese early diabetic animals.

Acknowledgements

Kathryn Gradin was supported by the Swedish Research Council, while Ulf was supported by the Danish Heart Foundation (99-2-2-35-22742) and the Danish Medical Research Council. We are grateful to Dr. K. Rudolf of Karl Thomae, Gmbh, Biberach, Germany, for the generous supply of BIBP 3226.

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