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Effects of phosphorothioated neuropeptide Y Y_1-receptor antisense oligodeoxynucleotide in conscious rats and in human vessels

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1 Metabolically stabilized (phosphorothioate) human and rat NPY Y_1 receptor oligodeoxynucleotides (ODNs) complimentary to the rat or human Y_1 mRNA were synthesized; [sense (rY_1-SODN, 5'-AATTCAACTCTGTTCTCC-3'), antisense (hY_1-ASODN, 5'-CCTGGGAAAATAATGTTG-3' and rY_1-ASODN, 5'-GGAGAACAGAGTTGAATT-3') and mismatches (hY_1-MMODN, 5'-CCTGAGATAA-TAAGGTTG-3' and rY_1-MM 5'-GTAGATCAGAGATGAAGT-3')] and used to modulate cardiovascular function *in vitro* in human vessels as well as *in vivo* in the rat.

2 The objectives of the experiments were to assess the influence of the NPY Y_1 receptor on vasomotor function human resistance arteries *in vitro* and to investigate the contribution of the NPY receptor system to cardiovascular haemodynamics *in vivo*.

3 Human subcutaneous resistance arteries removed from patients who underwent surgery for non-vascular diseases were incubated *in vitro* with the stabilized phosphorothioated hY_1-receptor ASODN or MMODN (10^{-7} to 10^{-5} M).

4 In human resistance vessels preincubated with hY_1-AS (10^{-7} to 10^{-5} M), the contractile response to NPY was significantly reduced in a dose-dependent fashion. No effects were observed in the hY_1-MMODN-incubated vessels at lower concentrations (10^{-7} M to 10^{-6} M).

5 The haemodynamic effects of the phosphorothioated rY_1-ASODN, SODN or MMODN were investigated in conscious rats during 48 h of continuous infusions. The continuous infusion with the rY_1-ASODN did not change MAP while the rY_1-SODN unexpectedly induced an early (10-20 h) increase in ambulatory MAP and the rY_1-MMODN a late (24-44 h) increase.

6 Contractile responses to NPY (2, 4, 8, 16 and 32 $\mu g\ kg^{-1}$) were significantly reduced in the rats treated with long-term infusion of rY_1-ASODN (2.1 mg $kg^{-1}\ h^{-1}$, i.v. infusion for 48 h) compared with animals treated with rY_1-SODN and MMODN, as well as animals treated with saline and glucose. Notably, the group infused with the rY_1-SODN showed an exaggerated response to tested doses of NPY.

7 We conclude that the incubation of human subcutaneous arteries with a metabolically stabilized 18 base pair hY1-ASODN and long-term infusion with a corresponding rY_1-ASODN attenuate NPY-induced vasoconstriction.

Keywords: Human artery; conscious rat; long-term infusion; antisense oligodeoxynucleotide; phosphorothioate (metabolically-stabilized)

Introduction

Neuropeptide Y (NPY) is a 36 amino acid peptide (Tatemoto *et al.*, 1982; Waeber *et al.*, 1988) present in the sympathetic nervous system (Lundberg *et al.*, 1983, 1986; Linton-Dahlöf *et al.*, 1989). Compared with noradrenaline (NA), it is a more potent vasoconstrictor, and in conscious rats, systemic administration of NPY produces a long-lasting increase in blood pressure due to a marked increase in total peripheral resistance (Zukowska-Grojec *et al.*, 1987). Specific binding sites for NPY in the peripheral cardiovascular system have been demonstrated in the porcine spleen (Lundberg *et al.*, 1988), rabbit (Chang & Lotti, 1988) and porcine aortic membranes (Shigeri *et al.*, 1991), as well as in rat cardiac ventricular membranes (Balasubramaniam *et al.*, 1990). Early experiments using a series of C-terminal peptide fragments of NPY led to the proposal of the existence of at least two different receptor subtypes; a postsynaptic (Y_1) and a presynaptic (Y_2) receptor (Wahlestedt *et al.*, 1986, 1987). Both Y_1- and Y_2-receptors appear to be involved in the regulation of vascular function. Research related to cardiovascular roles and other roles played by the NPY-receptor subtypes has been hampered by the lack of biologically useful and selective receptor antagonists. Consequently, our understanding of the role of this peptide in sympathetic vasoconstrictor co-transmission and its possible involvement in cardiovascular disorders is still limited.

Recently, antisense oligodeoxynucleotides to the rat Y_1-receptors have been used as a tool to explore some aspects of the functional role of the central Y_1 receptor (Wahlestedt *et al.*, 1993). In addition, Erlinge *et al.* (1993) reported that the NPY-induced vasoconstrictor effects *in vitro* in human mesenteric arterioles can be inhibited by incubation with antisense deoxynucleotides to NPY. A down-regulation of the receptor itself could thus provide a novel way of studying the effects of the NPY Y_1-receptor.

The objective of the present study was threefold: firstly, we wanted to establish whether it was possible to apply the technique of using a metabolically stabilized (phosphorothioate, Fisher *et al.*, 1993) NPY Y_1-receptor ASODN to modulate cardiovascular function *in vivo* in the rat. Secondly, we wanted to

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assess the contribution of the NPY receptor system to cardiovascular haemodynamics in the whole rat and thirdly, our aim was to investigate whether the metabolically-stabilized NPY Y_1-receptor ASODN could influence vasomotor function in human resistance arteries *in vitro*.

Methods

The experimental protocols used in this experiment were approved by the Animal Ethics Committée, University of Göteborg, and the Human Ethics Committée, University of Lund, Sweden.

The effect of phosphorothioate antisense oligodeoxynucleotide incubation on human subcutaneous arteries; vasomotor responses in vitro

Subcutaneous arteries were removed from patients who underwent surgery for non-vascular diseases. The arteries were dissected out from the abdominal region at the start of the operation and were cut into cylindrical segments (1–2 mm long). The segments were incubated in Dulbecco's modified essential medium (DMEM, Sigma U.S.A.) supplemented with streptomycin (100 μg ml^{-1}), penicillin (100 U ml^{-1}) with or without oligodeoxynucleotide (1 μM) for 72 h at 37°C in humidified 5% CO_2 and 95% air. The cylindrical segments were mounted on two L-shaped metal prongs (0.1 mm in diameter), one of which was connected to a Grass (FT03C) force displacement transducer attached to a Grass polygraph for continuous recording of isometric tension. The position of the other holder could be changed by means of a movable unit, thereby permitting fine adjustments of the vascular tension by varying the distance between the metal prongs. In some experiments, the resulting electrical signals from the transducers were amplified by Transbridge TBM4 amplifier, digitalized by Maclab TM analogue-digital converter and recorded by a Macintosh Plus computer.

The mounted specimens were immersed in temperature-controlled (37°C) tissue baths containing a buffer solution of the following composition (mM): NaCl 119, $NaHCO_3$ 15, KCl 4.6, $MgCl_2$ 1.2, NaH_2PO_4 1.2, $CaCl_2$ 1.5 and glucose 11. The solution was continuously gassed with 5% CO_2 in O_2 producing a pH of 7.4.

A tension of 4 mN was applied to the arterial segments and they were allowed to stabilize at this level of tension for 1.5 h. The contractile capacity of each vessel segment was examined by exposure to a potassium-rich (60 mM) buffer solution which had the same composition as the standard buffer solution, except that some of the NaCl was exchanged for an equimolar concentration of KCl. Only after two reproducible contractions had been achieved were the vessels used for further studies (variation less than 10%). After another 45 min rest period, NPY was added to the vessels in cumulative concentrations (10^{-10}–10^{-6} M). When the responses achieved peak levels, maximal contraction as a percentage of the 60 mM KCl contraction is expressed as E_{max}. The pD_2 values were calculated in Ragtime 3.1 using a formula that identifies the concentration of NPY which produces 50% of the maximal contraction.

Cardiovascular effects of intravenous infusion of NPY Y_1 oligodeoxynucleotides in vivo

Experimental animals Experiments were carried out in conscious male Sprague-Dawley (SD) rats (Møllegaard Hansen Avelslaboratorie A/S, Denmark) weighing 220–250 g. All the animals were maintained on standard rat pellets and tap water *ad libitum* and housed in the animal unit of the department in cages in groups of 5, ag 26°C, with 60% humidity and a light regimen of 05 h 00 min to 19 h 00 min.

Surgical preparation of conscious rats The animals were anaesthetized with pentobarbitone sodium (30 mg kg^{-1}, i.p.). The left carotid artery was cannulated with PE-50 tubing for blood pressure (BP) recordings and one PE-50 tube was inserted in the right jugular vein for infusion of the phosphorothioated oligodeoxynucleotide, 10% glucose, saline or the injection of various pressor agents. Immediately after the operative procedures, the individual cannulae were filled with heparin (100 U ml^{-1}) in saline, sealed and exteriorized at the nape of the neck.

Twenty to 24 h after the surgery, the rats were brought to the laboratory and placed individually in opaque plastic boxes (25.0 × 7.0 × 7.5 cm, i.d.). The boxes allowed the rats to move backward and forward and to turn around. The arterial catheter was connected to a Grass Model 7D polygraph via a P23 DC transducer (Grass Instruments, Quincy, Mass, U.S.A.) and pressure curves were continuously obtained on an ink printer. A cardiotachygraph triggered by the arterial pulse wave was used to record heart rate (HR). Systolic (SBP) and diastolic (DBP) blood pressure were calculated from the recordings.

Administration Phosphorothioated 18-mer ODNs complementary to the rat (r) or human (h) Y_1-mRNA were designed; rY_1-SODN, r(h)Y1-ASODN and r(h)Y1-MMODN. The stabilized ODNs (2.1 mg kg^{-1} h^{-1}), saline or 10% glucose were infused i.v. for 48 h in conscious normotensive Sprague-Dawley (SD) rats. The rats were allowed to become accustomed to their surroundings for 48 h, during which time the ASODN, SODN or MMODN or either 10% glucose or salaine was continuously infused. Basal BP and HR were monitored continuously.

When the infusion was complete, the pressor response curves of NPY as well as noradrenaline (NA), tyramine (Tyr) or endothelin-1 (ET-1) were tested. The pressor responses (i.a. MAP) were measured at peak effects.

Experimental protocols (i) In the set of experiments where *in vitro* vascular contractile responses to NPY (log concentration 10^{-10}–10^{-6} M) were studied in human subcutaneous arteries, the specimens were incubated 72 h with the hY1-ASODN and hY1-MMODN respectively in different concentrations (10^{-7} M, 10^{-6} M and 10^{-5} M). (ii) In *in vivo* experiments in which the effect of ASODN, MMODN, SODN or either glucose or saline on baseline blood pressure was evaluated, the respective agent was administered in an intravenous infusion of 2.1 mg kg^{-1} h^{-1} continuously over a 48 h period. Controls received saline or glucose (glucose or saline solution was infused at a rate of 0.397 ml h^{-1}). Each animal received only one treatment. The following protocol was used: 30 min after the animal was placed in the plastic box, the drug or saline was infused and the basal blood pressure (BP) recording was obtained during a 48 h period. Data registrations were obtained every hour during the first day and every 4 h during the second day.

(iii) At the end of the 48 h infusion, the pressor response to different doses of NPY, NA, tyramine (Tyr) and endothelin-1 (ET-1) was studied for each of the five groups of conscious rats. NPY was given as an injection in a dose of 2–32 μg kg^{-1}, NA given as 25–100 ng kg^{-1} in a bolus injection, Tyr given as 30–120 μg kg^{-1} in a bolus injection and ET administered at a dose of 400-800 pM kg^{-1} in a bolus injection. The increases in basal mean arterial blood pressure to the maximal peak height of the individual pressor responses were analysed.

When the infusion was complete, the first dose of NPY was given. Increasing doses of NPY were then given every 10 min to obtain a cumulative dose-response curve. NA administration was performed 20 min after the last dose of NPY was given, when the blood pressure recording was stable and baseline conditions were again present. A second bolus dose of NA was given 10 min later, followed by a third dose of NA after a 10 min interval. Tyr was given in the same way. The incremental doses of ET-1 were administered at 1 h intervals due to its protracted action. Each experimental group was composed of 6–12 animals.

Drugs

Methohexitone sodium (Brietal, Eli Lilly & Co, Indianapolis, IN, U.S.A.), pentobarbitone sodium (Mebumal vet Nord Vacc, Sweden), heparin (Lövens Läkemedel, Malmö, Sweden), neuropeptide Y (CRB, Cambridge, England), noradrenaline (Sigma, St Louis, MO, U.S.A.), tyramine (Sigma, St Louis, MO, U.S.A.), endothelin-1 (Sigma, St Louis, MO, U.S.A.) and phenylephrine (Sigma, St Louis, MO, U.S.A.) were used.

NPY and ET-1 were dissolved in saline containing 0.5% bovine serum albumin (BSA, Sigma, St. Louis, U.S.A.) to yield a solution of 40 μg/4 ml^{-1}.

Oligodeoxynucleotides

The phosphorothioate ODNs were constructed by replacing a non-bridging oxygen molecule of unmodified ODNs by a sulphur one at each base.

Rat ODNs The rat phosphorothioate 18 mer ODNs were as follows: (1) antisense ODN (rY1-ASODN: 5'-GGAGAACAGAGTTGAATT-3'; (2) mismatch ODN (rY1-MMODN): 5'-GTAGATCAGAGATGAAGT-3', with mismatches from rY1-ASODN underlined; (3) sense ODN (rY1-SODN): 5'-AATTCAACTCTGTTCTCC-3'.

Human ODNs The human phosphorothioate 18 mer ODNs were of the following compositions: (1) antisense (hY1-ASODN): 5'-CCTGGGAAAATAATGTTG-3'; (2) mismatch ODN (hY1-MMODN): 5'-CCTGAGATAATAAGGTTG-3', with mismatches from hY1-AS underlined; (3) sense (hY1-SODN): 5'-CAACATTATTTTCCCAGG-3'.

All the tested ODNs were synthesized on a Biosearch Cyclone DNA synthesizer. The phosphorothioated oligodeoxynucleotides were supplied by Midland Certified Reagent Company (Texas U.S.A.). After deprotection with 30% ammonium hydroxide, the ODNs were lyophilized and redissolved in water. These ODNs were run on 15% acrylamide gels to verify their size. The DNA sequence of the ODNs was checked for similarities against all human and rat DNA sequences present in the GENEMBL database.

The ASODN, MMODN and SODN were dissolved in 0.9% NaCl. The solution was prepared fresh before each experiment.

Statistical analysis

In vitro *studies:* The results are given below as a percentage of potassium-induced contraction, the maximum amount of contraction (E_{max}). The potency is expressed as pD_2(−log concentration of agonist inducing half-maximum contraction). The data are expressed as mean values ± s.e.mean.

Differences are assessed by Mann-Whitney U-test for calculations of statistical significance.

In vivo *studies:* All the values in the text, tables and figures are given as means ± s.e.mean. The statistics were calculated using the Macintosh Statview II programme on a Macintosh II computer. Group comparisons were performed using ANOVA with Student's unpaired or paired t test. A probability (P) level <0.05 was considered significant.

Results

The effects of oligodeoxynucleotide incubation in human subcutaneous arteries on the vasomotor responses induced by NPY

The contractile effects of NPY on human subcutaneous arteries were tested after 72 h incubation with phosphorothioated ASODN or MMODN in different concentrations: (10^{-7} M to 10^{-5} M) (Figure 1, Table 1).

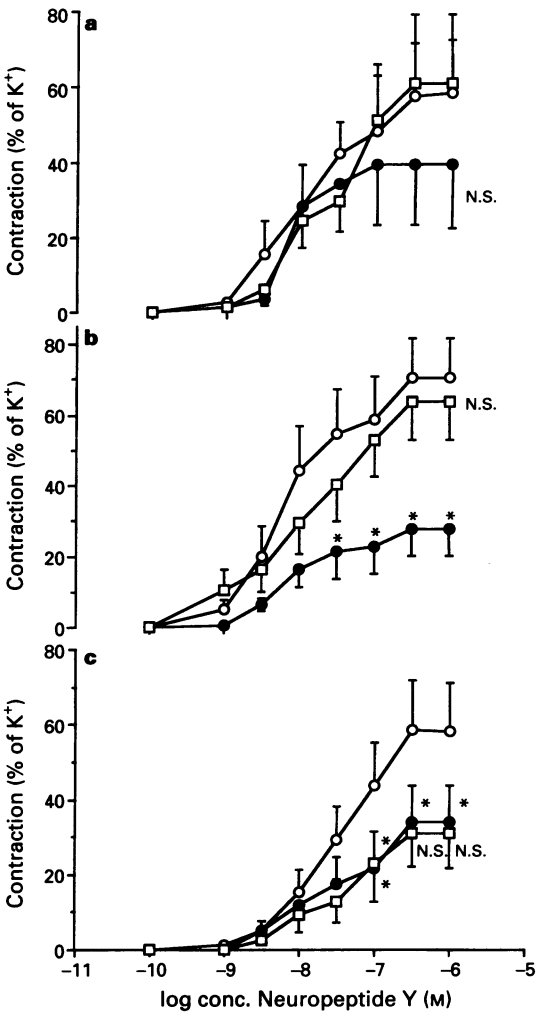

Figure 1 Contractile effects of NPY on human subcutaneous arteries incubated for 72 h with no oligodeoxynucleotide (○, control) or phosphorothioated antisense oligodeoxynucleotide (●, ASODN) or mismatches oligodeoxynucleotide (□, MMODN) in different concentrations 10^{-7} M (a, $n=5$), 10^{-6} M (b, $n=8$), and 10^{-5} M (c, $n=7$). Shown are means ± s.e.mean. Statistical comparison versus control group. *$P>0.05$.

Table 1 Contractile effect of NPY expressed as E_{max} and pD_2 on human subcutaneous arteries incubated for hours with phosphorothioted antisense oligodeoxyneucleotide (ASODN) or mismatches oligodeoxyneucleotide (MMODN) in different concentration

	E_{max} (%)		
	10^{-7} M	10^{-6} M	10^{-5} M
Control	58.2 ± 14.7	74.2 ± 12.3	58.8 ± 12.9
MMODN	60.8 ± 18.3	67.1 ± 11.2	31.5 ± 9.1
ASODN	39.3 ± 16.1	29.6 ± 8.8	34.4 ± 9.9
	pD_2		
	10^{-7} M	10^{-6} M	10^{-5} M
Control	7.90 ± 0.30	7.92 ± 0.24	7.57 ± 0.23
MMODN	7.61 ± 0.27	7.68 ± 0.30	7.56 ± 0.25
ASODN	8.30 ± 0.10	7.81 ± 0.27	7.52 ± 0.26

n = 5 for 10^{-7} M, n = 8 for 10^{-6} M, n = 10^{-5} M, means ± s.e.mean

The experiments were performed in parallel tissue baths and each segment was exposed to NPY only once. Despite the 72 h incubation in DMEM, with or without oligodeoxynucleotides, arteries responded to 60 mM KCl with powerful contractions, with no difference between any of the groups. In the arteries, the contraction elicited by KCl were 1.28 ± 0.23 Mn (no ODNs), 1.41 ± 0.23 mN (hY1-ASODN) and 1.54 ± 0.65 mN (hY1-MMODN).

The contractile response to NPY did not differ between the untreated group (no ODNs) and the ODN group (MMODN and ASODN) at the 10^{-7} M concentration. In the vessels preincubated with hY_1-ASODN in the concentration range 10^{-6} M to 10^{-5} M, the contractile response to NPY was significantly reduced (E_{max}), but no effect of pD_2 was observed (Figure 1, Table 1). At 10^{-6} M the MMODN did no inhibit NPY-induced contraction. However, at the higher concentration (10^{-5} M) the MMODN had inhibitory effects similar to the ASODN, although only significant at one concentration of NPY (10^{-7} M).

Haemodynamic effects of continuous infusion of phosphorothioated ASODN, SODN or MMODN in conscious rats

Control recordings were obtained in rats infused with either saline or 10% glucose. The changes in basal BP and HR were continuously monitored during a 48 h infusion in ASODN, SODN or MMODN.

Continuous infusion with the rY_1-ASODN did not change MAP compared with saline or 10% glucose (Figure 2). However, the rY_1-SODN induced an early (10–20 h) increase in ambulatory MAP and the rY_1-MMODN a late (24–44 h) increase. HRs were not altered.

Contractile responses to NPY and other pressor agents in conscious rats treated with ASODN, SODN, MMODN, glucose and saline

In order to test the nature of the inhibitory effects of ASODN on NPY induced pressor responses, different doses of NPY (2, 4, 8, 16 and 32 $\mu g\ kg^{-1}$) were given in the five groups. A significant dose-dependent pressor-response curve was induced by NPY in conscious SD rats in all groups (Figure 3). Compared with the saline, glucose, MMODN and SODN groups, the pressor response to NPY was significantly reduced in the rats given ASODN (2.1 mg $kg^{-1}\ h^{-1}$, i.v. infusion for 48 h). At the highest (32 $\mu g\ kg^{-1}$) dose of NPY, a significant difference compared to ASODN-treated rats was noted for animals treated with SODN, MMODN as well as the saline and glucose-treated animals (Figure 3). Unexpectedly, the animals given infusion with SODN, displayed an exaggerated response to all the tested doses of NPY. Correspondingly, the ASODN-treated rats displayed less pressor responses to low and high doses of NPY (Figure 3). Interestingly, the HR response was

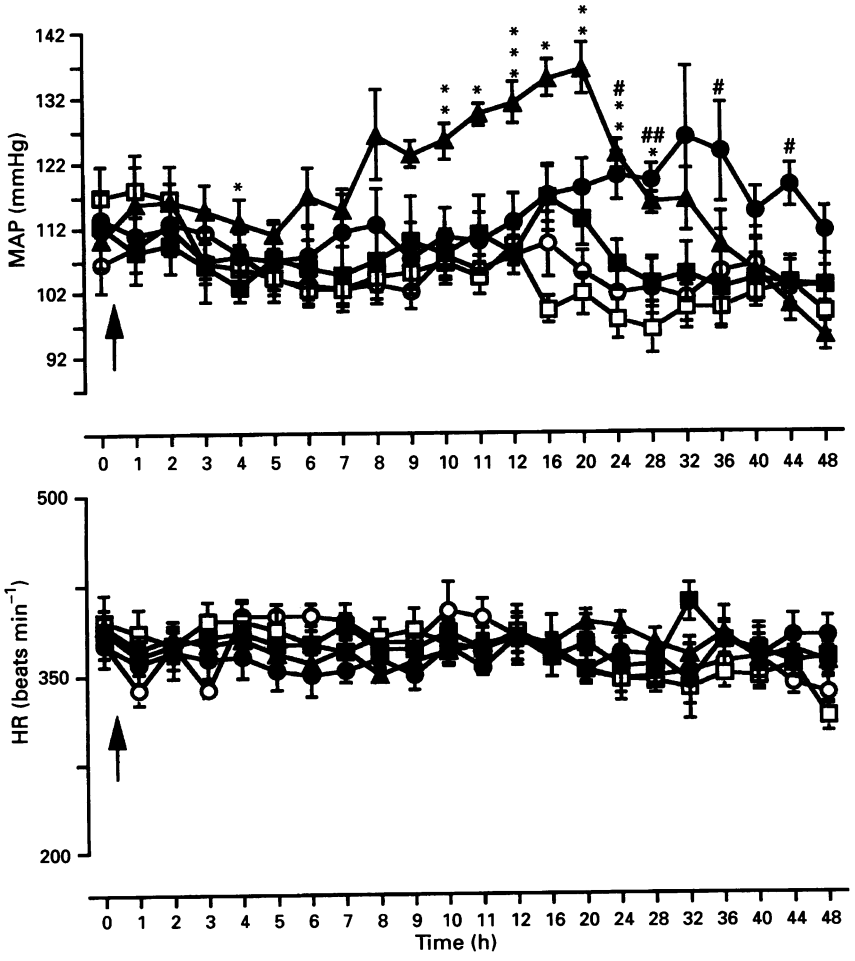

Figure 2 Basal mean arterial pressure (MAP) and heart rate (HR) responses in rats during 48 h continuous infusion of glucose (□), saline (○) or phosphorothioated mismatches oligodeoxynucleotide (●, MMODN), sense oligodeoxynucleotide (▲, SODN) or antisense oligodeoxynucleotide (■, ASODN) in a concentration of 2.1 mg $kg^{-1}\ h^{-1}$ infusion. Please note the non-linear time-scale. The means ± s.e.mean are shown. Significances indicated: * or # $P<0.05$; ** or ## $P<0.01$; and *** $P<0.001$. *indicates SODN *vs* ASODN, and #indicates MMODN *vs* ASODN.

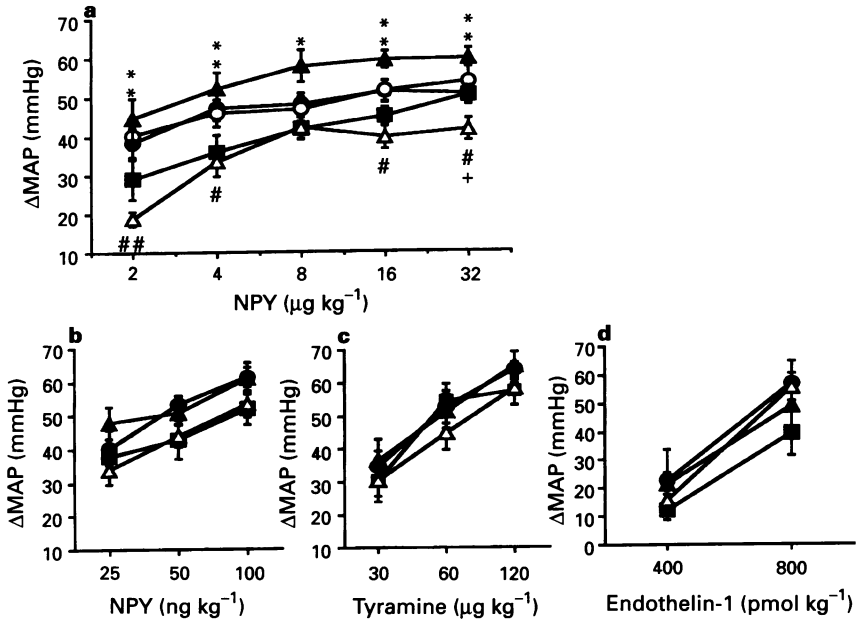

Figure 3 Pressor effects of NPY, NA, tyramine and endothelin-1 in intact conscious rats after 48 h continuous infusion of glucose (○), saline (●) or phosphorothioated mismatches oligodeoxynucleotide (■, MMODN), sense oligodeoxynucleotide (▲, SODN) or antisense oligodeoxynucleotide (△, ASODN) in a concentration of 2.1 mg kg^{-1} h^{-1} infusion. Shown are means ± s.e.mean of the change in mean arterial pressure (ΔMAP). Significances indicated: *, # or $^{+}$ $P<0.05$; ** or ## $P<0.01$. *indicates SODN *vs* ASODN; #saline or glucose *vs* ASODN and $^{+}$MMODN *vs* ASODN.

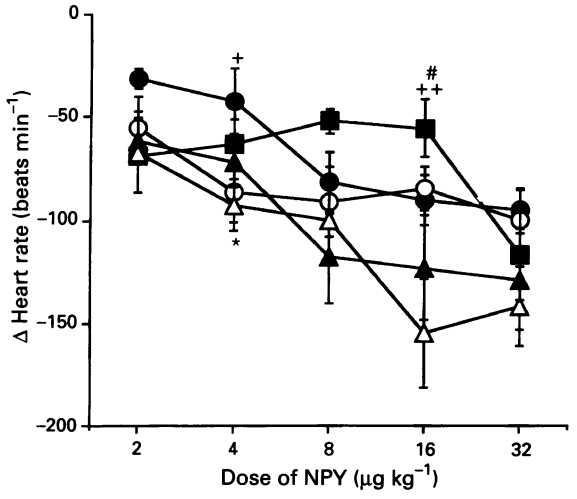

Figure 4 The effect of NPY on heart rate in conscious rats after 48 h continuous infusion of glucose (○), saline (●) or phosphorothioated mismatches oligodeoxynucleotide (■, MMODN), sense oligodeoxynucleotide (▲, SODN) or antisense oligodeoxynucleotide (△, ASODN) in a concentration of 2.1 mg kg^{-1} h^{-1} infusion. Shown are means ± s.e.mean of the change in heart rate (ΔHR), Significances indicated: *, # or $^{+}$ $P<0.05$; ** or $^{++}$ $P<0.01$; *indicates saline *vs* ASODN; #glucose *vs* ASODN and $^{+}$MMODN *vs* ASODN.

also significantly reduced in the animals infused with the ASODN (Figure 4), while it was increased in the rats pretreated with the MMODN.

In contrast to this, there were no significant reductions or rightward shifts in the pressor response curves to the NA (25, 50 and 100 ng kg^{-1}), Tyr (30, 60 and 120 μg kg^{-1}) or ET-1 (400 pmol kg^{-1} and 800 pmol kg^{-1}) in animals infused with ASODN or any of the other ODNs (Figure 3).

Discussion

The ASODN suppression of specific gene products was first used to modify viral and oncogene expression and was later introduced as a technique to down-regulate (knock-down) specific neurotransmitter receptors (Wahlestedt *et al.*, 1993). Recently, in addition to NPY Y_1, expression of several other receptor types have been down-regulated by antisense oligodeoxynucleotides such as AT_1, NMDA, ET_A, opioid and dopamine receptors (Wahlestedt, 1994; Adner *et al.*, 1994).

One of the problems with oligodeoxynucleotides is that they are readily degraded by enzymes in the blood and on cell surfaces. It is, however, possible to construct more stable oligodeoxynucleotides by replacing a non-bridging oxygen molecule by a sulphur one at each base (phosphorothioated nucleotides). In order to examine the role of NPY Y_1 receptor in the rat and the human peripheral circulation, we therefore designed 18 base antisense and mismatch phosphorothioated ODNs to a coding region near the human and rat NPY Y1 receptor NH_2-terminus.

The first report on the vascular actions of NPY Y_1 ASODN was provided by Erlinge *et al.* (1993) who studied incubated segments of human subcutaneous arteries and veins. They found that human NPY Y_1 receptor ASODN attenuated NPY-evoked vasoconstriction in both arteries and veins. In contrast, the corresponding SODN or 3-base mismatched MMODN did not influence NPY-induced vascular contraction. Since the pD_2 values did not differ between the vessels treated with ASODN and the MMODN or SODN, the results indicated a non-competitive receptor interaction as the result of down regulation of the human NPY Y_1 receptor by the ASODN. It is currently believed that the ASODN prevents the full expression of the NPY Y_1 receptor, thereby reducing the numbers of sarcolemma intact receptors.

In the present experiments, in order to modulate the biosynthesis of a human receptor, we designed an 18-base ASODN on the basis of the known mRNA sequence. Human subcutaneous arteries were incubated for 72 h and the contractile effect of NPY was examined. Our results showed that phosphorothioated ASODN (Fisher *et al.*, 1993)

(10^{-6} M – 10^{-5} M) concentration-dependently inhibited the contractile response induced by NPY (10^{-10} M – 10^{-6} M). The results also revealed that the stabilized ASODN inhibited the contractile effect of NPY without influencing the contraction induced by other pressor agents. This demonstrates that phosphorothioated ASODN (Fisher *et al.*, 1993) may also selectively inhibit the vasomotor effect of NPY at the Y_1-receptor site in human vasculature *in vitro*. It was therefore possible to apply the technique of using a metabolically-stabilized (phosphorothioate) NPY Y_1-receptor ASODN to modulate cardiovascular function *in vivo* in the rat.

In comparison with the hY_1-ASODN (10^{-6} M to 10^{-5} M) in human subcutaneous arteries, incubation with the MMODN (10^{-5} M) at high dose (10^{-5} M) also significantly reduced the contractile response to NPY. This was surprising since three nucleotides are interchanged in the ASODN sequence, so that it does not match the human Y_1 receptor mRNA sequence. It is possible that, in high concentrations, it could bind to the Y_1 receptor mRNA despite the mismatch. It is unlikely that the attenuation of the contractile response was due to nonspecific effects of the oligodeoxynucleotide, since the ASODN did not affect the response of the vessels to NA or K^+ depolarization.

Furthermore, and most importantly, our study demonstrates that long-term intravenous infusion of the phosphorothioated ASODN (Fisher *et al.*, 1993) significantly and selectively reduced the pressor response curve to NPY. In order to further characterize the profile of the ASODN on the NPY response, incremental doses of the agonist were tested after long-term administration of ASODN (2.1 mg kg^{-1} h^{-1}, i.v. infusion). Compared with rats infused with saline and 10% glucose and the rats treated with SODN and MMODN, ASODN induced a parallel reduction in maximal effect without a significant shift of the NPY pressor-response curve to the right, thus indicating that ASODN acts as a non-competitive inhibitor of NPY actions. During the 48 h infusion, we continuously monitored the behaviour in the five group of rats and no differences in behaviour were seen in any of the groups.

The present results show that long-term infusion with a metabolically-stabilized rY_1-ASODN attenuates the vasoconstriction in response to NPY in the conscious rat. Furthermore, incubation of human subcutaneous arteries with a metabolically-stabilized 18-base pair hY_1-ASODN induces concentration-dependent inhibition of the NPY response. This lends support to the notion that a metabolically-stabilized ASODN could represent a prototype for a new class of drugs capable of selectively antagonizing NPY Y_1-receptor actions.

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