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▶ **To cite this version:**

Andreas Birkenfeld, Michael Boschmann, Cédric Moro, Frauke Adams, Karsten Heusser, et al.. Beta-adrenergic and atrial natriuretic peptide interactions on human cardiovascular and metabolic regulation.. Journal of Clinical Endocrinology and Metabolism, Endocrine Society, 2006, 91 (12), pp.5069-75. 10.1210/jc.2006-1084 . inserm-00165273

HAL Id: inserm-00165273
https://www.hal.inserm.fr/inserm-00165273

Submitted on 25 Jul 2007

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Beta-Adrenergic and Atrial Natriuretic Peptide Interactions on Human Cardiovascular and Metabolic Regulation

Short title: ANP and beta-adrenergic receptors

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DISCLOSURE STATEMENT: The authors have nothing to disclose

Words (excluding abstract, references and figures): 3434
Words (abstract): 250,
2 tables, 5 figures

Acknowledgements: This work was supported in part by a Deutsche Forschungsgemeinschaft grant

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Abstract

Context: Atrial natriuretic peptide (ANP) has well known cardiovascular effects and modifies lipid and carbohydrate metabolism in humans.

Objective: To determine the metabolic and cardiovascular interaction of beta-adrenergic receptors and ANP.

Design: Cross over study, conducted 2004-2005

Setting: Academic clinical research center

Patients: Ten healthy, young, male subjects (BMI 24±1 kg/m²)

Intervention: We infused intravenously incremental ANP doses (6.25, 12.5, and 25 ng/kg/min) with and without propranolol (0.20 mg/kg in divided doses followed by 0.033 mg/kg/h infusion). Metabolism was monitored through venous blood sampling, intramuscular and subcutaneous microdialysis and indirect calorimetry. Cardiovascular changes where monitored by continuous ECG and beat-by-beat blood pressure recordings.

Main outcome measures: Venous NEFA, glycerol, glucose, insulin; microdialysate glucose, glycerol, lactate, pyruvate.

Results: ANP increased heart rate dose dependently. Beta-adrenergic receptor blockade abolished the response. ANP elicited a dose-dependent increase in serum non-esterified fatty acid and glycerol concentrations. The response was not suppressed with propranolol. Venous glucose and insulin concentrations increased with ANP, both, without or with propranolol. ANP induced lipid mobilization in subcutaneous adipose tissue. In skeletal muscle, microdialysate lactate increased while the lactate to pyruvate ratio decreased, both, with and without propranolol. Higher ANP doses increased lipid oxidation while energy expenditure remained unchanged. Propranolol tended to attenuate the increase in lipid oxidation.

Conclusions*:* Selected cardiovascular ANP effects are at least partly mediated by beta-adrenergic receptor stimulation. ANP induced changes in lipid mobilization and glycolysis are mediated by another mechanism, presumably stimulation of natriuretic peptide receptors

whereas substrate oxidation might be modulated through adrenergic mechanisms.

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Introduction

Atrial natriuretic peptide (ANP) is synthesized within the heart in response to myocardial stretch. ANP has well characterized cardiovascular effects including regulation of vascular tone, renal sodium handling, and myocardial hypertrophy.(1) Recent studies suggest that ANP also influences lipid and carbohydrate metabolism, thus providing a link between metabolic and cardiovascular regulation. ANP stimulates lipolysis *in vitro* in human adipocytes(2;3) and locally in subcutaneous adipose tissue.(2) Systemic ANP infusion in pharmacological (4;5) and physiological concentrations, (6) dose-dependently increase circulating non-esterified fatty acid (NEFA) and glycerol concentrations. Moreover, ANP might be involved in the control of lipid mobilization during exercise.(7;8) The in vitro studies suggest that ANP induced lipolysis is mediated through natriuretic peptide receptor A (NPrA) activation. NPrA stimulates hormone-sensitive lipase through the cGMP pathway.(3;9) Activated hormone-sensitive lipase cleaves triacylglycerides into NEFA and glycerol. Generally, lipolysis is regulated by the sympathetic nervous system. It is possible that metabolic responses to systemic ANP infusions are mediated through the sympathetic nervous system rather than NPrA activation. Indeed, the ANP mediated reduction in blood pressure may cause baroreflex mediated increases in sympathetic nervous activity. Larger ANP concentrations increase norepinephrine concentrations, heart rate,(6;10) muscle sympathetic nerve activity,(10) and regional vascular resistance.(11) Yet, ANP may also have a direct sympatholytic effect.(10;12) In a previous study, local glycerol concentrations were not reduced by local beta-adrenergic receptor blockade applied via microdialysis.(5) However, local beta-adrenergic receptor blockade does not exclude the possibility that ANP-induced metabolic changes are secondary to reflex-mediated beta-adrenergic receptor stimulation. We tested the hypothesis that ANP induced changes in glucose and lipid metabolism, in particular adipose tissue lipolysis, are secondary to beta-adrenergic receptor stimulation.

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Materials and Methods

Subjects. We studied 10 healthy men (age 32±2 yr, body mass index, 24±1 kg/m^2, waist to hip ratio, 0.82 ±0.05). They received no medications. Human subcutaneous adipose tissue for *in vitro* experiments was obtained from 6 moderately overweight women undergoing plastic surgery (34±1 years, BMI 26±1 kg/m^2).Written informed consent was obtained before study entry. The institutional review board approved all studies including sampling of adipocytes from surgical specimen.

Protocol. Subjects abstained from smoking, alcohol ingestion, caffeine-containing beverages, and vigorous exercise 48 hours before the experiments. We conducted all experiments in the morning after an overnight fast. Probands remained supine throughout the experiment. ANP was infused on two separate days, either with or without the non-selective beta-adrenergic receptor blocker propranolol in a cross over fashion. The washout phase between experiments was at least seven days. After a resting phase of 30 minutes, we obtained baseline indirect calorimetric measurements over a 30 minute period. Then, we removed the ventilated hood, and started incremental human ANP infusions (Merck Bioscience AG, Clinalfa, Switzerland). We infused ANP at rates of 6.25, 12.5, and 25 ng/kg/min for 45 minutes each. These infusion rates have previously been shown to provide physiological to pathophysiological venous ANP concentrations in humans.(6) The duration of each infusion was sufficient to attain the maximal ANP mediated lipolytic effect of each dose.(5) We repeated indirect calorimetry measurements during the last 30 minutes of each ANP-infusion step. When the non-selective beta-adrenergic receptor blocker propranolol was applied in combination with ANP, it was given as follows: 0.2 mg/kg intravenously in four divided bolus doses before ANP infusion followed by continuous propranolol infusion with 0.033mg/kg/h throughout the experiment. These doses have previously been shown to completely inhibit isoproterenol induced lipolysis.(13) Venous blood and microdialysis

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samples were collected at baseline and every 15 minutes of ANP infusion.

Instrumentation: Before resting phase, two venous catheters (Vasocan, 20G, B. Braun, Germany) were placed in large antecubital veins of both arms. Infusions and blood sampling were performed on contra lateral arms. One microdialysis probe (CMA60) each was inserted into abdominal subcutaneous adipose tissue (SCAT) and into femoral skeletal muscle (quadriceps femoris, vastus lateralis) as described previously.(14;15) Respiration and ECG were measured continuously (Cardioscreen, Medis GmbH). Beat-by-beat finger blood pressure (Finapres, Ohmeda, USA) was recorded continuously throughout experiments. Brachial arterial blood pressure (Dinamap, Critikon, USA) was determined automatically every 5 minutes contra laterally to the infusion side. We used a ventilated hood to monitor O_2 consumption and CO_2 production (DeltatracII, Datex Ohmeda, Germany) by indirect calorimetry to assess energy expenditure and substrate oxidation rates. Whole-body carbohydrate- and fat-oxidation rates were estimated using stoechiometric equations(16).

Microdialysis: Details of the microdialysis-technique are described elsewhere.(14;15) Briefly, before insertion of the probes, we applied a local anesthetic (lidocaine) either as a cream for adipose tissue (EMLA, Astra GmbH, Germany) or as a subcutaneous injection for muscle (Xylocitin 1%, Jenapharm GmbH, Germany). After probe insertion, we started the tissue perfusion with lactate free Ringer solution (Serumwerk Bernburg AG, Germany) at a flow rate of 2 µL/min. The solution was supplemented with 50 mmol/L ethanol (EtOH, B. Braun Melsungen AG, Germany). CMA/60 microdialysis catheters and CMA/102 microdialysis pumps (both from CMA Microdialysis AB, Sweden) were used. A 60 minute period was allowed for tissue recovery and for baseline calibration. Two 15 minute dialysate fractions were collected at baseline.

Analytical methods. Venous and in vitro glycerol concentrations were determined by an ultra sensitive radiometric method as described previously(6) Venous NEFA were assayed with an enzymatic method (Wako kit, Unipath), insulin concentrations were measured using a

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radioimmunoassay (Sanofi Diagnostics Pasteur, France). Ethanol concentrations in perfusate (inflow) and dialysate (outflow) were measured by enzymatic techniques.(17) Based on Fick's principle, a decreased dialysate-to-perfusate ratio (ethanol ratio) indicates an increased blood flow and vice versa.(18;19) For simplicity, the term ethanol ratio is substituted for the term ethanol outflow/inflow ratio. Dialysate glucose, lactate, pyruvate, and glycerol concentrations were measured with a CMA/600 analyzer (CMA Microdialysis AB, Sweden). The *in situ* recovery, assessed by near-equilibrium dialysis at a flow rate of 0.3 µl/min, was about 30% in adipose tissue and 50% in skeletal muscle for all four metabolites.

Heart rate variability: The electrocardiogram was analog to digital converted at 500 Hz using the Windaq Pro+ software (Dataq Instruments Inc., USA). The RR intervals (time between subsequent R waves in the electrocardiogram) were detected off-line using a program written by André Diedrich (Vanderbilt University, Nashville, USA) based on PV-wave software (Visual Numerics Inc., USA). We analyzed heart rate variability in the time domain using standard techniques. In addition, we calculated spectra of R-R interval time series in the high- and in the low frequency range using Fast Fourier transformation based algorythm.(20)

In vitro experiments: Adipocytes were isolated by collagenase digestion as described previously (3) After digestion, the suspension was filtered (210-µm filter) and washed 3 times with PBS. Then, adipocytes were brought to a suitable dilution (2000-3000 cells/100 µl) into Krebs Ringer Bicarbonate Hepes 10 mmol/L buffer containing glucose (5.55 mmol/L) and 20 mg/mL of BSA at pH 7.4. Then, adipocytes were incubated during 90 minutes with increasing propranolol concentrations, namely 10^{-6}, 10^{-5} and 10^{-4} mol/L, in presence of 100 nmol/L isoproterenol or ANP at 37°C under gentle shaking at 120 cycles/min in a water bath. After incubation, 50 µl of medium were taken to measure glycerol and total lipids were extracted gravimetrically as described previously (3).

Calculations and statistics: All data are expressed as mean±SEM. Repeated-measures

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ANOVA testing was used for multiple comparisons. Bonferoni's post hoc test was performed, when $P<0.05$. A value of $P<0.05$ was considered significant.

Results

All subjects tolerated ANP and propranolol infusions well. Three probands had to be excluded from the analysis after experiments had been performed. One proband showed raised venous glucose concentrations at baseline on one of the study days suggesting that he was not in the fasted state. In the second proband, NEFA measurements at baseline differed more than twofold on both study days. In the third subject, several venous measurements including NEFA could not be obtained at baseline and on different time points during drug infusion. In our analysis, we only included seven subjects with a full data set.

Hemodynamic responses: With ANP infusions, heart rate increased dose dependently from 56±1 beats per minute (bpm) at baseline to 72±2 bpm at the highest ANP infusion rate ($p<0.01$). With the combination of ANP and propranolol, heart rate was 56±1 bpm at baseline and did not increase over time ($p<0.001$ for the propranolol effect) (figure 1a). Heart rate variability is displayed in detail in table 1. Heart rate variability in the time domain decreased substantially during ANP infusion. Furthermore, we observed a reduction in heart rate variability in the low frequency and in the high frequency domain with incremental ANP infusion. Propranolol attenuated ANP induced changes in heart rate variability. However, the ANP induced reduction in heart rate variability was not fully suppressed with propranolol. With ANP infusion, blood pressure was 118±3/61±3 mm Hg at baseline and 114±4/59±3 mm Hg at the highest ANP infusion rate (ns). With the combination of ANP and propranolol, blood pressure was 116 ±3/68±3 mm Hg at baseline and 106±4/59±3 mm Hg at the highest ANP infusion rate ($p=0.06$). Mean arterial blood pressure is displayed in figure 1b.

Venous measurements: With ANP infusions, venous NEFA and glycerol concentrations increased dose dependently (Fig.2 a, b). The response was not attenuated with

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propranolol. ANP increased glucose, without or with propranolol (Fig. 1 c). Without propranolol, insulin was 4.2±0.6 µU/mL at baseline, 4.80±0.9 µU/mL at an infusion rate of 6.25 ng/kg/min, 5.3±1.2 µU/mL at an infusion rate of 12.5 ng/kg/min, and 5.7±0.6 at the highest ANP infusion rate (p=0.06 vs baseline). With the combination of ANP and propranolol, venous insulin concentrations were 4.7 µU/mL at baseline, 4.2±0.5 mU/mL at the lowest ANP-infusion rate, 4.3±0.2 µU/mL at an infusion rate of 12.5 ng/kg/min, and 5.7±0.6 µU/mL at the highest infusion rate (ns for propranolol effect).

Microdialysis: Adipose tissue ethanol ratio and glucose concentrations are given in table 1. In abdominal subcutaneous adipose tissue (SCAT), the ethanol ratio tended to decrease with and without propranolol. SCAT glucose and lactate concentrations increased without ($p<0.05$) and with propranolol ($p<0.01$) at the highest ANP infusion rate (Fig. 3b) (ns for propranolol effect). With ANP, the maximal increase in SCAT glycerol concentrations was 36% ($p < 0.05$) with ANP, and 54% ($p<0.01$) with the combination of ANP and propranolol at the highest ANP infusion rate (ns for propranolol effect) (Fig. 3a).

In skeletal muscle, the ethanol ratio did not change during ANP infusions with and without propranolol. Similarly, ANP did not change dialysate glucose concentrations. Yet, dialysate lactate concentrations maximally increased by 25% ($p<0.001$) with ANP and by 32% ($p<0.05$) with ANP and propranolol (Fig. 3d) (ns for propranolol effect). Dialysate pyruvate concentration increased to a maximum of 530% ($p<0.001$) with ANP and 670% ($p<0.001$) with ANP and propranolol ($p<0.001$) (ns for propranolol effect). The lactate to pyruvate ratio decreased with ANP from 103±43 at baseline to 24±3 ($p<0.001$) at the highest ANP infusion rate and from 354±272 at baseline to 43±14 ($p<0.001$) at the highest ANP infusion rate with propranolol (ns for propranolol effect). The numerical difference in the lactate to pyruvate ratio with and without propranolol was solely explained by a single proband with an unusually low pyruvate measurement on propranolol. Skeletal muscle dialysate glycerol concentrations did not change throughout the ANP infusion (Fig. 3c).

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Indirect calorimetry: Resting energy expenditure did not change significantly with ANP or with the combination of ANP and propranolol (figure 4 a). The respiratory quotient tended to increase above baseline at an ANP infusion rate of 6.25 ng/kg/min, but decreased below baseline as infusion rate was further increased (figure 4 b). Accordingly, carbohydrate oxidation rate increased by 45% above baseline at an ANP infusion rate of 6.25 ng/kg/min, and decreased by 30% below baseline at an ANP infusion rate of 25 ng/kg/min ($p<0.05$ vs 6.25 ng/kg/min). Without propranolol, lipid oxidation rate tended to decrease below baseline at an ANP infusion rate of 6.25 ng/kg/min, while it increased to a maximum of 34% above baseline with the highest ANP infusion rate ($p<0.05$ vs baseline). The biphasic response of lipid oxidation rate was attenuated with propranolol. We calculated the amplitude of the change in lipid oxidation rate as the difference between maximal lipid oxidation rate and minimal lipid oxidation rate in each subject. The amplitude was 13±1.3 g/6h without propranolol and 7±1.9 g/6h with propranolol ($p<0.05$).

In vitro experiments: Figure 5 illustrates changes in glycerol concentrations with isoproterenol and ANP in the presence and in the absence of propranolol in human mature adipocytes. Isoproterenol increased glycerol release substantially. The response was abolished with 1 µmol/L propranolol. Larger propranolol concentrations decreased glycerol concentrations below the baseline value. In contrast, the ANP driven glycerol release from isolated adipocytes was not attenuated with propranolol. The relative decrease in glycerol with ANP and the highest propranolol concentration is equivalent to the decrease in glycerol values below baseline with isoproterenol and the highest propranolol concentration.

Discussion

We conducted *in vitro* and *in vivo* experiments to study the interaction of ANP and beta-adrenergic receptors on human metabolism and cardiovascular regulation. The main finding of our study is that propranolol attenuated the ANP induced changes in heart rate and

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in heart rate variability. In contrast, ANP induced changes in lipolysis and carbohydrate metabolism were not attenuated with propranolol. Initially, we tested the effect of isoproterenol and ANP on *in vitro* lipolysis, both in the presence and in the absence of propranolol. Adipocytes exhibited a strong lipolytic response to isoproterenol and to ANP. Moderate propranolol concentrations abolished the lipolytic response to isoproterenol. In contrast, ANP mediated lipolysis was maintained even with high propranolol concentrations. The observation further supports the idea that beta-adrenergic receptor agonists and ANP induce lipolysis through distinct receptor and post receptor mechanisms.(3;9) ANP activates hormone sensitive lipase through a cGMP-dependent pathway.(9) Our results suggest that the pathway is sufficient to sustain lipolysis even during near complete beta-adrenergic receptor blockade. On the other hand, ANP-induced lipid oxidation tended to be responsive to beta-adrenergic receptor blockade in this study.

Data from *in vitro* studies may not reflect the *in vivo* situation. For instance, a cell-based experiment cannot test ANP induced changes in sympathetic activity. Sympathetic activity could be raised through baroreflex mechanisms compensating for ANP induced vasodilatation and volume loss from the intravascular space. With higher ANP concentrations, norepinephrine concentrations increase.(10) In addition, in this study, ANP infusion increased heart rate in part through beta-adrenergic receptor stimulation. The ANP induced reduction in heart rate variability was not fully suppressed with propranolol. The observation of decreased high frequency component during infusion suggests that ANP may have caused also a withdrawal of parasympathetic activity towards the heart in addition to the sympathetic activation.

Possibly, ANP increased sympathetic outflow to adipose tissue. To address this issue, we applied incremental intravenous ANP concentrations in humans, both in the absence and in the presence of near-complete systemic beta-adrenergic receptor blockade with propranolol. Propranolol in doses applied here abolishes the lipolytic response to local

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isoproterenol infusion.(13) Similar to earlier studies,(6) ANP increased circulating NEFA and glycerol concentrations. In the present study, systemic beta-adrenergic receptor blockade did not attenuate the response. To further address the interaction between ANP and beta-adrenergic mechanisms on lipolysis, we assessed adipose tissue and skeletal muscle metabolism using microdialysis. We observed a tissue-specific ANP effect on lipolysis. Local concentrations of glycerol in adipose tissue increased markedly with ANP. The response was not abolished with propranolol. In contrast, ANP in skeletal muscle did not increase glycerol concentrations. Our observations, together with previous studies using local beta-adrenoreceptor blockade,(5) indicate that ANP-induced lipolysis in humans cannot be explained by stimulation of beta-adrenergic mechanisms.

Our findings do not completely exclude the possibility that other, non-sympathetic, mechanisms are involved in the lipolytic action of ANP. Insulin inhibits lipolysis. ANP tended to increase venous insulin concentrations without and with propranolol. ANP may reduce the hepatic deactivation of insulin, thus, increasing circulating insulin concentrations.(21) In addition, the increase in plasma insulin concentrations could be secondary to a net glucose release from the liver.(22) Clearly, an increase in lipolysis with ANP cannot be explained by changes in insulin concentration. Other lipolytic agents, such as ACTH, GH, and cortisol were not altered by ANP in previous investigations.(23)

NEFA that are released through lipolysis may undergo different metabolic pathways. They may be re-esterified and stored as triacylglycerides or they may be oxidized. ANP had a biphasic effect on lipid oxidation rate with a marked increase in lipid oxidation rate at higher doses. Propranolol attenuated ANP mediated changes in lipid oxidation rate. Thus, stimulation of beta-adrenergic receptors appears to modulate ANP induced lipid oxidation. Non-selective beta-adrenergic receptor blockade impairs lipid oxidation rate during physical exercise.(24) However, exercise induced lipid oxidation during beta-blockade is normalized when circulating NEFA concentrations are restored through intralipid-heparin infusion.(25)

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Decreased NEFA availability alone cannot explain the attenuation in ANP induced lipid oxidation during beta-adrenoreceptor blockade. It is possible that beta-adrenergic tone and ANP are essential cofactors converging on the same metabolic pathway. Given its important role in the regulation of substrate oxidation and its activation by adrenergic receptors, (26) AMP-activated protein kinase might be involved in the metabolic interaction of the sympathetic nervous system and ANP.

Besides its effect on lipid turnover, ANP elicits complex changes in carbohydrate metabolism. Similarly to earlier reports,(5;6) venous glucose and insulin levels increased concentration dependently with ANP infusion. Reflex-mediated adrenergic receptor activation could contribute to the response. Epinephrine infusions increase blood glucose by stimulating hepatic glycogenolysis and the hepatic and renal gluconeogenesis. However, this explanation is unlikely because near complete beta-adrenergic receptor blockade did not alter the ANP mediated increase in blood glucose concentration in our study. An alternative explanation is that ANP induced hepatic gluconeogensis.(22) Finally, increased NEFA concentrations may have led to a secondary increase in gluconeogenesis and a reduced cellular uptake of glucose.(27)

Changes in circulating glucose concentration were associated with changes in glucose utilization at the tissue level. In skeletal muscle and in adipose tissue, ANP increases lactate production.(6) Furthermore, pyruvate concentration increased in skeletal muscle. Thus, ANP stimulated glycolysis. The reduction in pyruvate to lactate ratio suggests that in skeletal muscle, a greater proportion of glucose undergoing glycolysis was fed into the Krebs cycle. No change in microdialysate glucose concentration in the setting of increased venous glucose concentration and unchanged tissue blood flow with ANP is further evidence for increased muscular glucose uptake and metabolism. These responses were not inhibited by beta-adrenergic receptor blockade. However, we cannot exclude involvement of alpha-adrenergic mechanisms. Alpha-1 adrenergic receptor stimulation augments glucose uptake and

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glycolysis in human adipose tissue.(28) An alternative explanation for the increase in glucose, pyruvate and lactate might be inhibition of pyruvatdehydrogenase activity by NEFA.(29) With an increased proportion of glucose carbon being directed into the Krebs cycle, one would expect to see an increase in systemic carbohydrate oxidation rate. We observed no change or even reduction in carbohydrate oxidation rate with ANP. It is possible that an increase in skeletal muscle glucose oxidation was masked by opposing metabolic changes in other organs, such as the liver. Indeed, in mice, ANP reduced hepatic lactate and pyruvate production due to increased gluconeogenesis.(22) The liver has a greater contribution to resting energy expenditure than skeletal muscle.

The sympathetic nervous system is generally regarded as the principle regulator of human lipolysis.(30) However, *in vitro* ANP was a more potent lipolytic agent than the beta-adrenoreceptor agonist isoproterenol.(2) Interestingly, ANP stimulates lipolysis in human and monkey adipocytes but not in rat, mouse, rabbit, hamster, and dog, adipocytes.(31) The phenomenon may reflect differences in NPr expression in adipose tissue between primates and other species. The ratio of the NPrA to the NPrC, the ANP clearance receptor, is approximately 100 fold smaller in rodent compared with human adipocyte membranes.(31) Clearance of natriuretic peptides by NPrC may attenuate the lipolytic effect. In obese patients, adipose tissue NPrC expression is increased, while natriuretic peptide concentrations are decreased. (32;33) In contrast, mice with a genetically non-functional NPrC, are thin and lack normal fat deposits.(34) Heart failure patients, in whom natriuretic peptide concentrations are elevated up to 20 fold, are prone to loss of adipose tissue, lean mass, and bone mass. The condition is called cardiac cachexia and carries a poor prognosis.(35) ANP might play an important role in this setting. Insulin resistance is another common metabolic abnormality in heart failure patients.(36) ANP induced NEFA release could conceivably contribute to insulin resistance.(27) Natriuretic peptide concentrations positively correlate with NEFA concentrations in patients with heart failure and coronary heart disease.(37;38) In

these patients, increased NEFA concentrations are associated with increased mortality.(38) Recently, natriuretic peptide infusions (nesiritide) have been espoused in heart failure therapy. While improving symptoms and hemodynamic parameters, nesiritide may have a neutral or even negative effect on prognosis.(39) Whether or not natriuretic peptide mediated changes in lipid and/or carbohydrate metabolism are important in this regard deserves further study.

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Figure Legends

Figure 1: a) Heart rate with increasing ANP dosages with and without the non-selective beta adrenergic receptor blocker propranolol b) Mean arterial blood pressure with ANP and with the combination of ANP and propranolol. (BB = Beta-adrenergic receptor blockade). ** p<0.01, *** p<0.001 compared with baseline measurement (post hoc analysis), **p<0.001** = comparison between curves by two-way ANOVA.

Figure 2: Venous NEFA (a), glycerol (b) and glucose concentrations (c) with incremental ANP infusion. ANP was infused with and without propranolol. P values in italics refer to comparisons versus baseline. (BB = Beta-adrenergic receptor blockade) (Conversion factors to convert to metric units are as follows: 0.028 for NEFA, 0.009 for glycerol, 18.02 for glucose) *=p<0.05, ** p<0.01, *** p<0.001 compared with baseline measurement (post hoc analysis), ns = no significant difference between curves by two-way ANOVA.

Figure 3: Microdialysate glycerol concentrations in adipose tissue (a) (ADIPOSE) and skeletal muscle (b) (MUSCLE) with incremental ANP infusion in the presence and absence of propranolol. Microdialysate lactate concentrations in adipose tissue (c) and skeletal muscle (d) (BB = Beta-adrenergic receptor blockade, Conversion factors to convert to metric units are as follows: 0.009 for glycerol, 9.0 for lactate) *=p<0.05, ** p<0.01, *** p<0.001 compared with baseline measurement (post hoc analysis), ns = no significant difference between curves by two-way ANOVA.

Figure 4: Relative change in energy expenditure (a) and respiratory qutient (b) with incremental ANP infusion with and without propranolol. (BB = Beta-adrenergic receptor

blockade). ***=p<0.001 compared with baseline measurement (post hoc analysis), **ns** = no significant difference between curves by two-way ANOVA.

Figure 5: Glycerol release from mature human adipocytes in vitro with 100 nmol/L isoproterenol (a) and 100 nmol/L ANP (b). Testing was conducted in the presence and in the absence of propranolol (concentrations are given in 10^x mol/L).

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Tables

Table 1: Heart rate variability.

	ANP infusion rate [ng/kg/min]			
	baseline	**6.25**	**12.5**	**25**
pnn50 [%]				
ANP	35±5	36±5	22±5	11±4*
ANP&BB	39±5	46±5	38±10	31±6
hf rri [msec²]				
ANP	1130±280	900±160	810±190	240±60*
ANP&BB	1240±270	1140±190	920±170	860±220
lf rri [msec²]				
ANP	970±250	1050±170	1060±170	490±100
ANP&BB	1230±300	1630±360	1290±250	1520±390

pnn50 = proportion of successive normal-to-normal intervals differences greater than 50 msec, hf rri = RR variability in the high frequency range, lf rri = RR variability in the low frequency range, *=$p<0.05$ ANP vs ANP&propranolol

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Table2: Adipose tissue microdialysis.

ANP infusion rate (ng/kg/min)	**Ethanol Ratio** ANP	ANP & Prop	**Glucose (mmol/L)** ANP	ANP & Prop
0	0.57±0.07	0.56±0.04	0.84±0.11	1.05±0.12
6.25	0.53±0.06	0.52±0.06	0.88±0.12	1.21±0.15
12.50	0.53±0.06	0.52±0.02	0.97±0.15	1.38±0.18
25	0.52±0.06	0.51±0.02	1.06±0.16*	1.39±0.18**
ANP vs ANP&Prop	ns		ns	

* = p<0.05 vs baseline, **=p<0.01 compared with baseline measurements.

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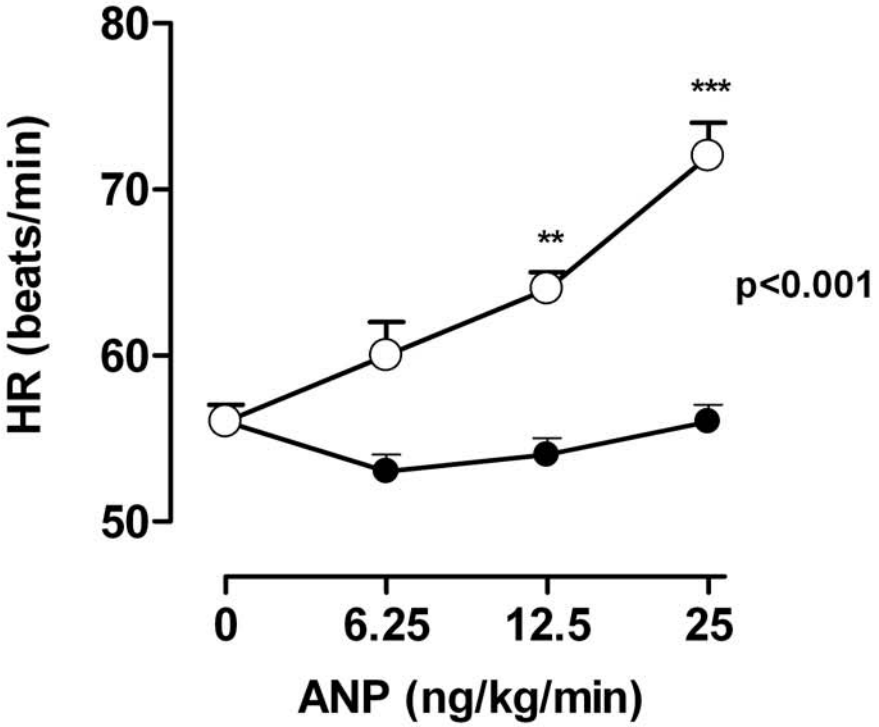

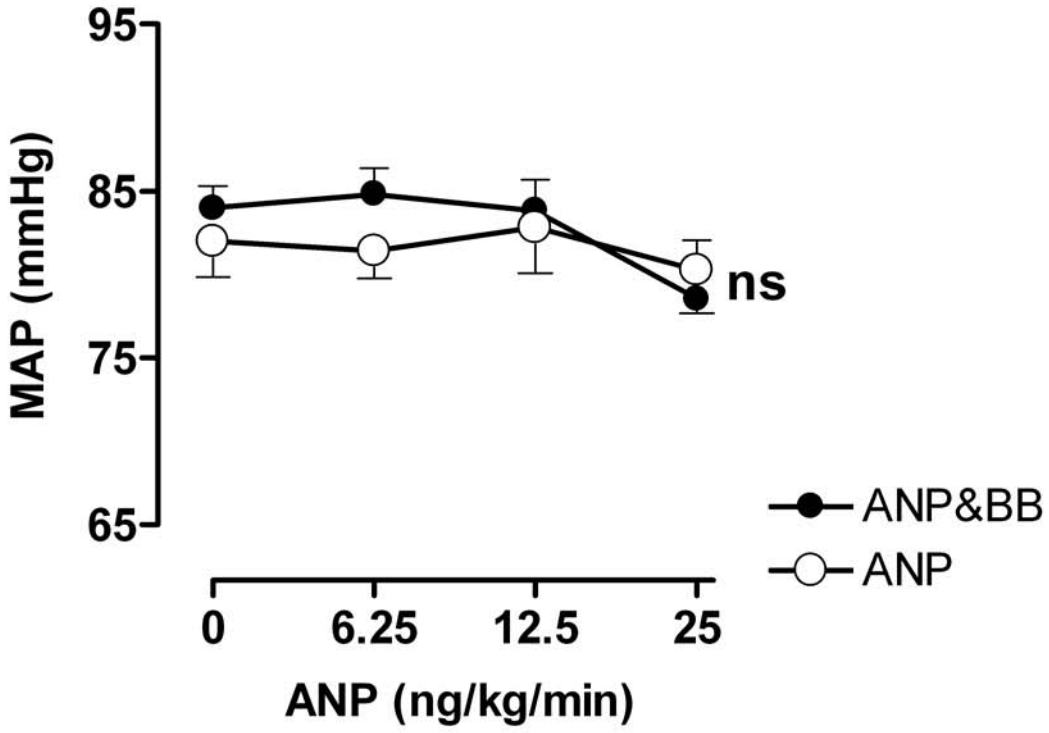

figure 1 a,b

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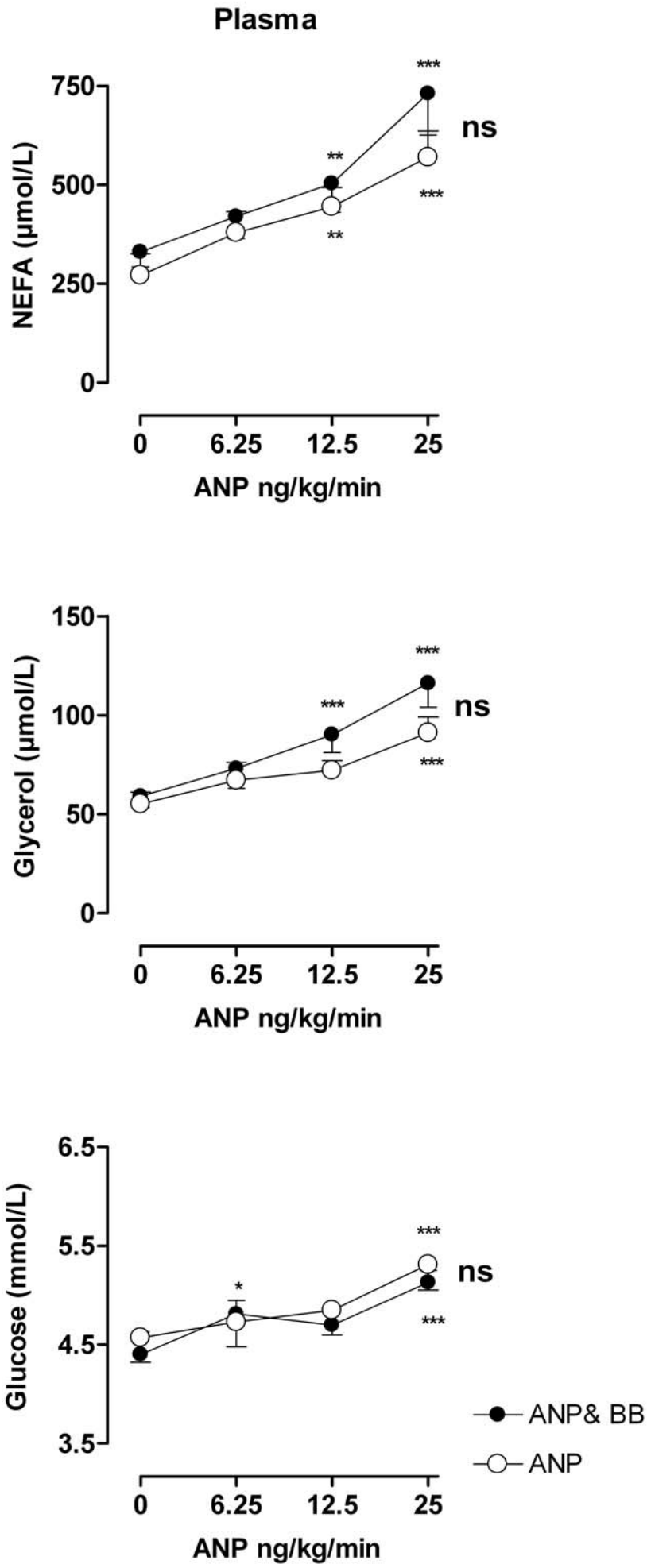

figure 2 a-c

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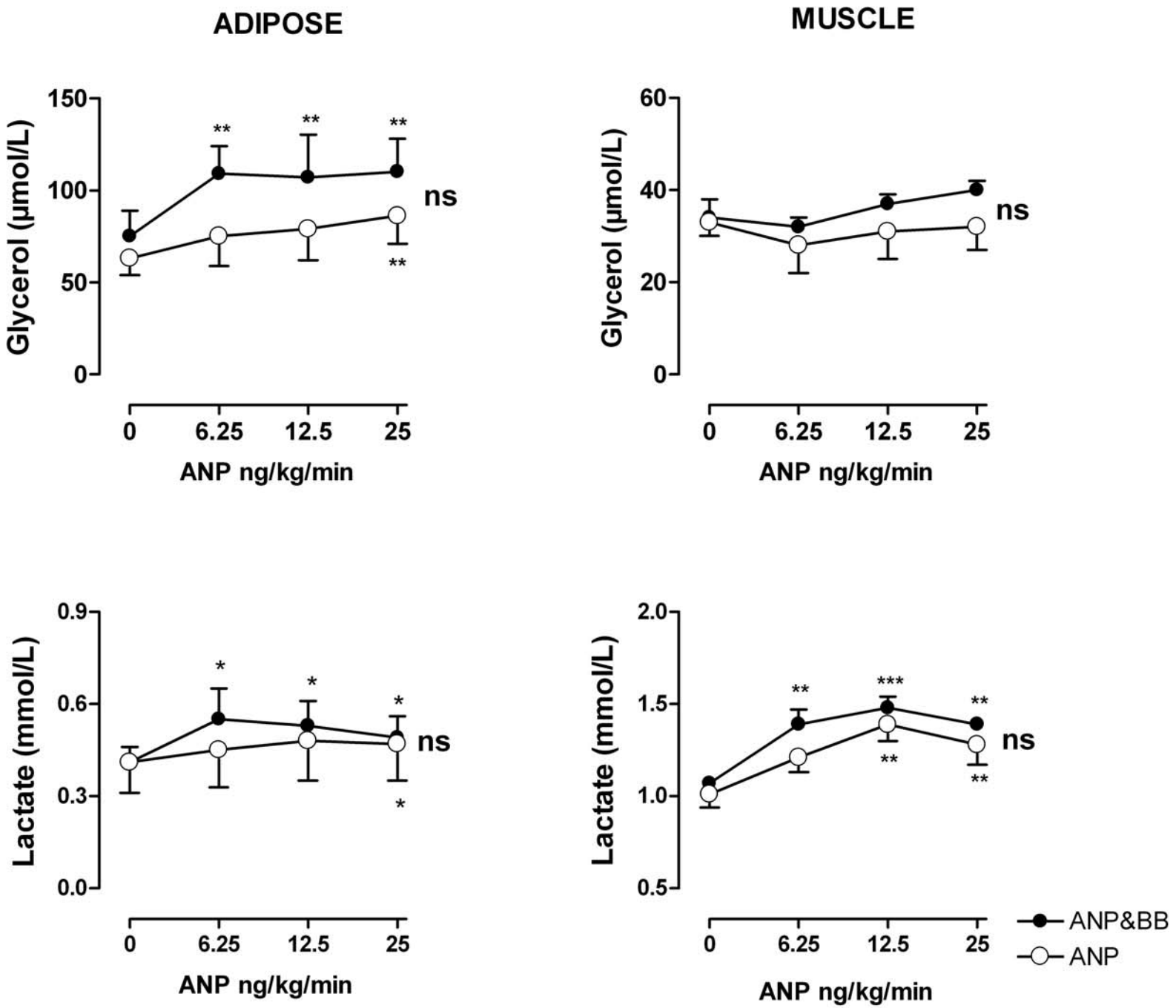

figure3 a-d

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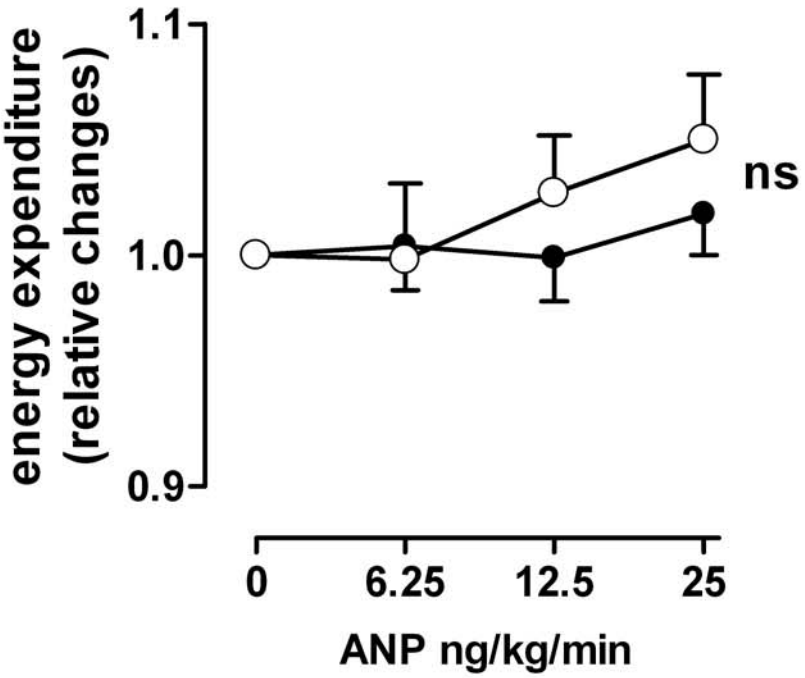

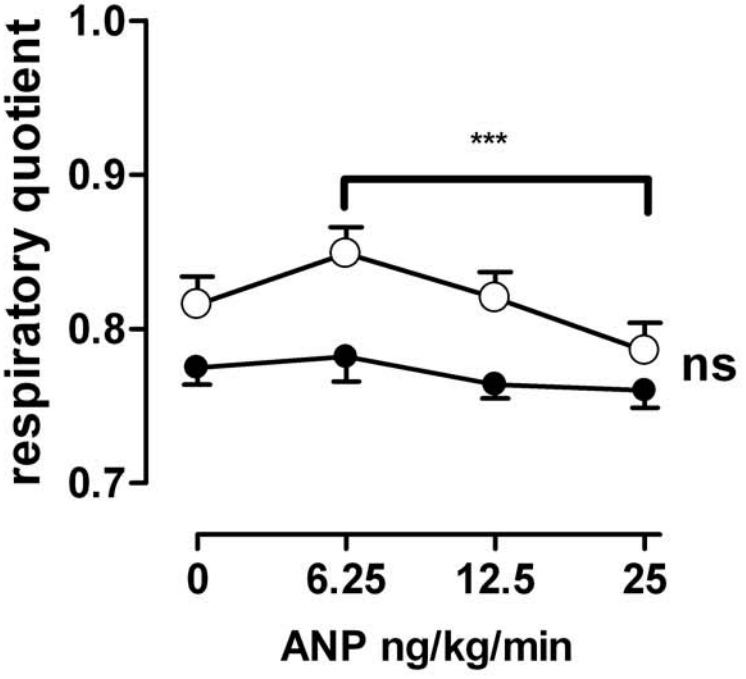

figure 4 a,b

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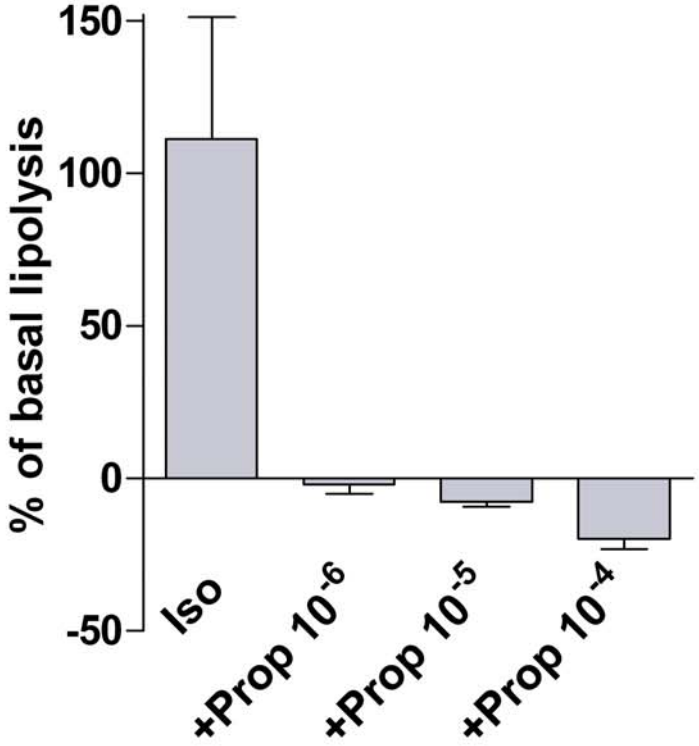

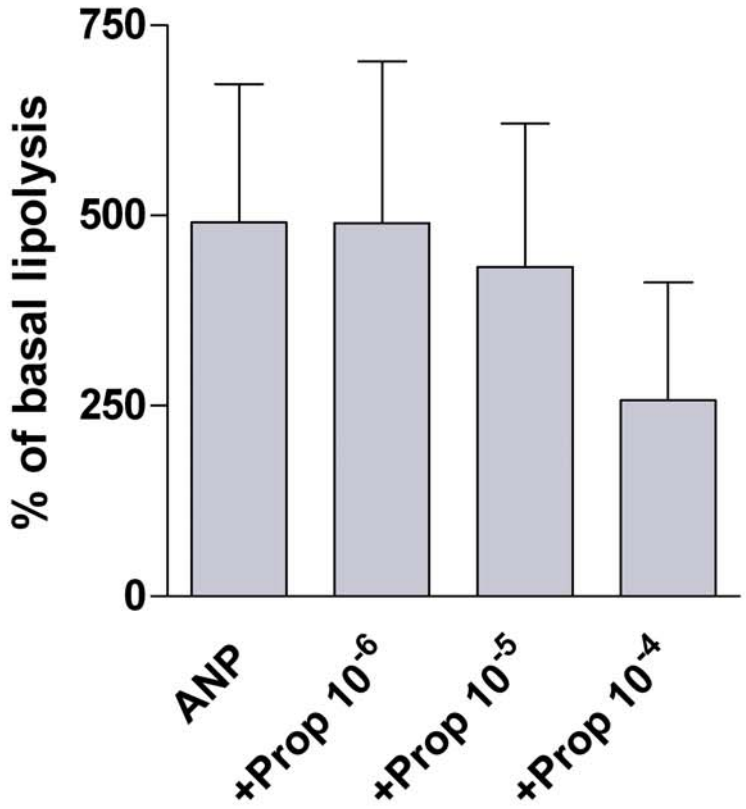

figure5 a,b