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Basic study

Differential effects of atrial and brain natriuretic peptides on human pulmonary artery: *In vitro* study

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Abstract

BACKGROUND

The prevalence of cardiovascular diseases especially heart failure continues to rise worldwide. In heart failure increasing levels of circulating ANP and BNP are associated with a worsening of heart failure and a poor prognosis.

AIM

In a previous study we found that BNP acts as a partial agonist in human pulmonary arteries (PA) and we hypothesised that a high concentration of BNP would inhibit relaxation to ANP.

METHODS

Pulmonary arteries were dissected from disease free areas of lung resection and PA rings of internal diameter 2.5–3.5mm and 2mm long were prepared. PA rings were mounted in a multiwire myograph and a basal tension of 1.61gf was applied. After equilibration for 60 minutes rings were pre-constricted with 11.21μM $PGF_{2\alpha}$ (EC_{80}) and concentration response curves were constructed to vasodilators by cumulative addition to the myograph chambers.

RESULTS

Although both ANP and BNP were found to vasodilate the pulmonary vessels, ANP is more potent than BNP. pEC50 of ANP and BNP were 8.96±0.21 and 7.54±0.18 respectively and the maximum efficacy (E_{max}) for ANP and BNP was -2.03gf and -0.24gf respectively. After addition of BNP the E_{max} of ANP reduced from -0.96gf to -0.675gf (p=0.28).

CONCLUSION

BNP could be acting as a partial agonist in small human PAs and inhibits relaxation to ANP. Elevated levels of circulating BNP could be responsible for worsening of decompensated heart failure. This finding could also explain the disappointing results seen in clinical trials of ANP and BNP analogues for the treatment of heart failure.

Key Words: Heart failure, ANP, BNP, in-vitro, humans

Core Tip: This study demonstrated that both ANP and BNP vasodilate isolated human pulmonary artery rings and BNP acts as a partial agonist and inhibits the effects of ANP. The finding that addition of BNP inhibits the effects of ANP suggests that BNP does act as a partial agonist and could be advancing the progression to decompensated heart failure.

Hussain A, Bennett RT, Tahir Z, Isaac E, Chaudhry MA, Qadri SS, Loubani M, Morice AH. The differential effects of Atrial and Brain Natriuretic Peptides on Human Pulmonary Artery: In Vitro Study. *World J Cardiology* 2019;

INTRODUCTION:

Decompensated Heart failure is a worldwide health issue that is associated with considerable morbidity and mortality. (1, 2) Despite the development of several device and medical based therapies over the past few decades, the rate of rehospitalisation and early death has not improved significantly.(3)

Natriuretic peptides (NP) family, consist of three structurally interrelated vasoactive peptides and was initially discovered by Bold et.al. in 1981 (4). The family includes atrial natriuretic peptide (ANP), brain natriuretic peptides (BNP) and C-type natriuretic peptide (CNP) that are mainly secreted by cardiac myocytes in response to wall stress (5, 6). ANP and BNP act via guanylyl cyclase linked natriuretic peptide receptor-A (NPR-A) whereas CNP activates a related cyclase, natriuretic peptide receptor-B (NPR-B) (7). ANP and BNP exert their beneficial effects by reducing systemic and pulmonary vascular resistance and by increasing natriuresis and diuresis (8). In addition to their haemodynamic effects, natriuretic peptides attenuate vascular smooth muscle proliferation and cardiac hypertrophy (9, 10). They also inhibit the synthesis of growth factors, by counteracting the effects of the renin-angiotensin system, that are involved in the development of pulmonary hypertension (11).

In-vitro studies on pulmonary arterial rings and isolated lung models have shown that ANP and BNP infusion induced pulmonary vasodilation by reducing pulmonary vascular resistance (12, 13). But in heart failure increasing levels of circulating ANP and BNP are associated with a worsening of heart failure and a poor prognosis(14). The aim of this study is to evaluate whether BNP does act as a partial agonist and inhibit the effects of ANP.

MATERIALS AND METHODS:

Study patients:

Local research ethics committee and institutional (Hull & East Yorkshire Hospitals NHS Trust) Research and Development Department approval was obtained for the use of lung specimens and surplus lung tissue from patients undergoing elective lobe or lung resection for cancer. Patients gave written consent for the use of surplus tissue for research purposes.

In accordance with the recommendations of the human tissue act (2004) 127 and the conditions of the local ethics committee approval the donor patient was anonymous to the researcher.

Tissue collection:

Excess segments of pulmonary artery were obtained from patients undergoing lobectomy and the sample was immediately transferred to the lab in Krebs-Henseleit solution after resection. After removal of connective tissue the pulmonary artery (PA) sample was divided into 2 mm long rings. The small size pulmonary vessels of internal diameter of 2-4 mm were used for these experiments.

Experimental protocol:

A multiwire myograph system was used for measurement of isometric tension. Under physiological conditions (37°C, 21% O_2), PA rings were mounted in Krebs Henseleit solution. A resting tension of 1.61 gf applied that was calculated from earlier experiments (15) and the vessels were left to equilibrate for 60-90 minutes. After equilibration vessels were preconstricted with 11.21µM PGF2α (EC_{80}, calculated from earlier experiments (16)) and concentration response curves were constructed to ANP and BNP by cumulative addition to the myograph chambers.

In another set of experiments once the vessels tension reached a plateau after preconstriction with PGF2α, 300 nM of BNP was added and the vessels left for 30 minutes. When a stable resting tension was achieved concentration response curves were constructed to ANP. Vessels were then washed for 30 minutes and the whole experiment repeated again without the addition of BNP.

Active tension was calculated in gram force (gf) as maximum tension at plateau (gf) – resting tension (gf). The maximum efficacy (E_{max}) for each agent was determined in gf and expressed as gf/mm internal diameter of each vessel (to take into account the variability in PA ring diameter). The Integrity of the endothelium was confirmed with 1µM Acetylcholine and KCl was added to check the viability. Vessels that did not constrict to KCl were excluded from the study. Figure 1 showed the schematic representation of myogrpah setup for measuring the isometric tension.

Chemicals used:

A 5%CO2/21%O2 air container was bought from BOC Limited Company (Surrey, UK). ANP, BNP and PGF2α were sourced from Tocris Bioscience, Acetylcholine was bought from Sigma-Aldrich and rest of the reagents were purchased from Thermo Fisher Scientific. For every experiment fresh solutions were prepared according to supplier recommendations and control responses were obtained when needed.

Statistical Analysis:

The experiments results are presented as mean ± SD (standard deviation) and the number of PA rings used represented by n. The concentration of agent required to attain 50% of maximum response response (Agonist EC50 concentrations) was measured using Graph Pad Prism version 7.00 (Graph Pad Software, USA). pEC50 represents the negative logarithm of the molar EC50 concentration and for all analysis significance was taken as $p < 0.05$.

RESULTS:

A total of 35 PA rings were obtained from 15 patients. The internal diameter of pulmonary arteries ranged from 2.5 – 3.5 mm. 9 rings were not included as they didn't response to KCl.

Concentration dependent effect of ANP and BNP on human pulmonary arteries:

ANP and BNP caused a concentration dependent relaxation of pulmonary arteries pre-constricted to $PGF_{2\alpha}$ with a pEC_{50} of 8.96±0.21 and 7.54±0.18 for ANP and BNP respectively [Fig 2]. The maximum efficacy (E_{max}) for ANP and BNP was -2.03 gf and -0.24 gf respectively.

Another set of experiments was conducted to determine whether a high concentration of BNP would inhibit relaxation to ANP. After addition of BNP the E_{max} of ANP was reduced by 30 % from -0.96 gf to -0.675 gf ($p=0.28$, $n=11$)[Fig 3].

Concentration response curve of ANP induced pulmonary vasodilation:

All 08 PA rings exposed to ANP showed relaxation which was maximal at 100nM (log -7.0M), while the ANP concentration used was ranged from 3pM

– 1µM. The recorded hill slope was 0.75 ± 0.5 and EC20, EC50 and EC80 were 0.17nM, 1.105nM and 7.01nM respectively.

Concentration response curve of BNP induced pulmonary vasodilation:

Increasing concentration of BNP from 1nM – 1µM was used to assess the reactivity of 07 PA rings to BNP. At 300nM concentration of BNP the vasodilatory response of vessels was maximal. The calculated hill slope was -1.818 ± 2.55.

Cumulative Concentration response curve of ANP and BNP induced pulmonary vasodilation:

16 PA rings from 07 patients and increasing concentration of ANP from 1pM – 1µM was used to evaluate the cumulative vasodilator effect of ANP and BNP on pulmonary vasculature. 05 rings were excluded, as they didn't respond to KCl. After equilibration all vessels were pre-constricted to 11.21 µM PGF2α (EC80) and once stable plateau relaxation was attained, the increasing concentration of ANP was added to determine its effect on PA vessels.

The PA rings than washed for 60 minutes and were pre-constricted again with 11.21 µM PGF2α (EC80). A single dose of 300 nm of BNP was added and left for 30 minutes. Once a plateau was achieved, by cumulative addition to the myograph chambers the concentration response curve of ANP was performed. The addition of BNP reduced the Emax of ANP by 30 % (from -0.96 gf to -0.675 gf)

DISCUSSION:

In this study we demonstrated for the first time that 1) both ANP and BNP vasodilate isolated human pulmonary artery rings and 2) that BNP acts as a partial agonist and inhibits the effects of ANP. The finding that addition of BNP inhibits the effects of ANP suggests that BNP does act as a partial agonist and could be advancing the progression to decompensated heart failure.

The circulating concentration of ANP, BNP and CNP is low in healthy individuals but it is elevated in heart failure patients, although to variable

degree (e.g. CNP elevated to a lower extent than it counterparts) (17, 18). In patients with HF, circulating concentration of BNP exceeds that of ANP; this consistency of response and high dynamic range makes bioassays for plasma BNP more useful than ANP (19, 20). This might be due to the fact that BNP is also a marker of cardiac remodelling (21). Previous studies have shown that in heart failure (HF) patients, BNP and NT-pro BNP (N-terminal pro b-type natriuretic peptide) are independent predictors of cardiovascular mortality, worsening HF and need for hospitalization (22-24). Although BNP and NT-pro BNP have prognostic value their therapeutic value is inconclusive in HF patients (25).

In early 21st century U.S. Food and Drug Administration (FDA) approved the use of Nesiritide (recombinant endogenous BNP) for heart failure patients(26). However several subsequent studies demonstrated that Nesiritide is associated with worsening renal function and increased risk of death (27). A randomized, double blind, placebo-controlled, ASCEND-HF (Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart Failure) trial concluded that nesiritide showed no substantial improvement in dyspnoea or clinical outcomes (28). Another double blinded, multicentre, randomized clinical trial, ROSE-AHF (Renal Optimization Strategies Evaluation - Acute Heart Failure) enrolled 360 patients. The study was designed to evaluate the use of low dose nesiritide with the view that there would be less side effects and substantial therapeutic effects. However, the study failed to provide significant evidence in support of routine use of nesiritide in heart failure patients (29).

Although natriuretic peptides (NPs) are always attractive therapeutic target for heart failure treatment their use is limited by inadequate clinical efficacy. It is thought that Neprilysin; a protease produced by kidney that cleaves various vasoactive compounds including BNP, activity is increased in heart failure (30). In heart failure increasing levels of circulating ANP and BNP are associated with a worsening of heart failure and a poor prognosis. This raised the suspicion that BNP might act as a partial agonist and inhibit the effects of

ANP, as shown in this study. These findings could also explain the disappointing results seen in clinical trials of ANP and BNP analogues for the treatment of heart failure. Further studies are needed to confirm the findings of this study, which raises the possibility that selective BNP antagonists could be of more clinical benefit than BNP agonists in the treatment of heart failure.

LIMITATIONS:

Our study had several limitations. It was a laboratory-based project that carried out in a control setting, which might not truly reflect the in-vivo environment. The therapeutic dose and the dose provided in the experiments may differ. We also used a pre-constrictor, $PGF_{2\alpha,}$ and since the potency of the agent depends on the pre-constrictor, other pre-constrictors need to be analyzed and compared. The full potential of the study needs to be backed by a double-blinded randomized control trial.

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