C-type natriuretic peptide signalling and cardiovascular disease

Adrian Hobbs

Professor of Cardiovascular Pharmacology
William Harvey Research Institute
Barts & The London School of Medicine & Dentistry
Queen Mary University of London
Charterhouse Square
London EC1M 6BQ
Natriuretic peptide family

ANP
28aa peptide

BNP
32aa peptide

CNP
22aa peptide
Biological actions of natriuretic peptides

**ENDOCRINE**
- Thirst
- Vasopressin
- Sympathetic

**PARACRINE**
- ANP (atria)
- BNP (ventricles)

**Na⁺/H₂O loss**
- Aldosterone

**RELAXATION**
- ANP
- BNP
- CNP
Natriuretic peptide receptor (NPR) subtypes

NPR-A  ANP  BNP  GTP  cGMP  GTP

NPR-B  GTP  cGMP  GTP

NPR-C  CNP  NPR-

Lysosome

Natriuretic peptide receptor (NPR) subtypes

Barts and The London
School of Medicine and Dentistry
Endothelium-dependent dilatation

Blood vessel lumen

Endothelium

Smooth muscle cell

- PGI₂
- NO
- EDHF

- ATP → cAMP → RELAXATION
- ATP → cGMP → RELAXATION
- Na⁺/K⁺-ATPase → 2K⁺ / 3Na⁺

HYPERPOLARISATION

Barts and The London
School of Medicine and Dentistry
Vascular pharmacology of CNP

Endothelial Cell

ACh, Shear Stress, IL-1β, TNFα, TGFβ

CNP

NPR-B

NPR-C

cGMP
GTP

Smooth Muscle Cell

Relaxation
CNP and EDHF activate NPR-C!

- CNP and EDHF cause relaxation.
- CNP and EDHF are potentiated by HS-142-1, an NPR-A/B antagonist.
- CNP and EDHF are inhibited by M372049, an NPR-C antagonist.

Rat isolated mesenteric arteries
Vascular pharmacology of CNP

Endothelial Cell

CNP

Leukocytes
Platelets

M372049

NPR-B

GIRK

Hyperpolarisation

Smooth Muscle Cell

NPR-C

ERK 1/2

Proliferation
A cytoprotective role for CNP in ischaemia-reperfusion (I/R) injury

**Graph:**
- ΔCPP (mmHg) vs. CNP (nmol)
- Control
- + Ba²⁺ + ouabain

**Infarct size (% of total wet weight):**
- Control
- + CNP (30nM)
- + CNP (30nM; @ rep)

**Legend:**
- Control
- + Ba²⁺
- + ouabain

**Diagram:**
- Langendorff isolated heart
- Ligature
- Non-ischaemic zone
- Ischaemic zone

Barts and The London
School of Medicine and Dentistry
A physiological role for CNP?

1. Interesting pharmacodynamic profile!

2. Presence of CNP in the endothelium suggests it has tissue localisation & functional remit to preserve vascular homeostasis
   - Acts as an EDHF in mesenteric and coronary arteries
   - Prevents the activation of leukocytes & platelets
   - Maintains integrity of blood vessel wall (regulates EC and VSMC proliferation)

3. Paucity of selective pharmacological interventions targeting natriuretic peptide signalling
   - Difficult to define a physiological function
Development of an endothelium-specific CNP knockout

Endogenous *Nppc* locus

![Diagram showing restriction sites and exons](image)

**Figure a:** Survival rate over age in mice with different genotypes.
**Figure b:** Image of mice with different genotypes.
**Figure c:** Naso-anal length over age in wild-type and mutant mice.
**Figure d:** Naso-anal length over age in wild-type and mutant mice.

*BamHI*  *SacI*  *ATG*  *Stop*  *SspI*  *Nhel*  *Exon 2*  *Exon 3*

**Dwarfism and early death in mice lacking C-type natriuretic peptide**


40/15 4021 | PNAS | March 27, 2001 | vol. 98 | no. 7
Development of an endothelium-specific CNP knockout

Endogenous \textit{Nppc} locus

Targeting vector

Targeted \textit{Nppc} locus

\textbf{ES cell injection}
Development of an endothelium-specific CNP knockout

Endogenous Mouse CNP Locus

LoxP (Floxed) Mouse

ecCNP KO Mouse

Barts and The London
School of Medicine and Dentistry
Development of an endothelium-specific CNP knockout

CNP mRNA expression

Plasma [CNP]

WT  ecCNP KO  WT  ecCNP KO

***  ***

(12.5mg/kg; i.p.; 14 hrs)

Barts and The London
School of Medicine and Dentistry
Vasoconstrictor responses are unaltered in ecCNP KO vessels

**Aorta**

- Log [Phenylephrine] M
- % Contraction
- pEC$_{50}$
  - WT: 6.91 ± 0.02
  - KO: 6.87 ± 0.02
- ns

**Mesentery**

- Log [Phenylephrine] M
- % Contraction
- pEC$_{50}$
  - WT: 5.72 ± 0.06
  - KO: 5.71 ± 0.08
- ns

- Log [U46619] M
- % Contraction
- pEC$_{50}$
  - WT: 7.97 ± 0.03
  - KO: 7.87 ± 0.02
- ns

- Log [U46619] M
- % Contraction
- pEC$_{50}$
  - WT: 7.78 ± 0.10
  - KO: 7.59 ± 0.16
- ns
Endothelium-dependent relaxation is impaired in resistance arteries of ecCNP KO mice

**Aorta**

NO, PGI₂ & EDHF-dependent relaxation

**Mesentery**

EDHF-dependent relaxation

NO, PGI₂ & EDHF-dependent relaxation
Blood pressure is elevated in ecCNP KO mice

Circadian Rhythm

MABP (24hr Mean)

WT
ecCNP Het
ecCNP KO

Genotype

MABP (mmHg)

Light
Dark
Light

WT
ecCNP Het
ecCNP KO

Genotype

MABP (mmHg)
Loss of endothelial CNP results in increased leukocyte rolling.

**Basal Rolling**

- WT basal: 5 cells/min
- KO basal: 25 cells/min

**IL-1β Treatment**

- WT IL-1β: 15 cells/min
- KO IL-1β: 50 cells/min
Loss of endothelial CNP: platelet function?

Impedance aggregometry

Platelet-leukocyte aggregates

---

**Impedance aggregometry**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Collagen (3µg/ml)</th>
<th>Collagen (10µg/ml)</th>
<th>PAR4-AP (300µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>![WT bar graph]</td>
<td>![WT bar graph]</td>
<td>![WT bar graph]</td>
</tr>
<tr>
<td>ecCNP KO</td>
<td>![ecCNP KO bar graph]</td>
<td>![ecCNP KO bar graph]</td>
<td>![ecCNP KO bar graph]</td>
</tr>
</tbody>
</table>

**Platelet-leukocyte aggregates**

<table>
<thead>
<tr>
<th>Condition</th>
<th>% Platelet-leukocyte aggregates</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>![WT bar graph]</td>
</tr>
<tr>
<td>ecCNP KO</td>
<td>![ecCNP KO bar graph]</td>
</tr>
</tbody>
</table>
Increased platelet & endothelial P-selectin expression in ecCNP KO mice

**Platelet P-selectin expression**

**Endothelial P-selectin expression**

- WT
- ecCNP KO
Accelerated atherogenesis in CNP KO mice

Oil Red O Staining (Lipid)

**Genotype**

**Lesion Size (% Entire Aorta)**

- ecCNP WT/ApoE KO
- ecCNP KO/ApoE KO

**Lesion Size (% Aortic Arch)**

- ecCNP WT/ApoE KO
- ecCNP KO/ApoE KO

**Lesion Size (% Abdominal Aorta)**

- ecCNP WT/ApoE KO
- ecCNP KO/ApoE KO

**Lesion Size (% Thoracic Aorta)**

- ecCNP WT/ApoE KO
- ecCNP KO/ApoE KO

(12 week high-fat diet)
CNP/ApoE double knockout mice have larger atherosclerotic plaques

- Plaque Area (% of total lumen area)
  - CNP WT/ApoE KO vs CNP KO/ApoE KO: **

- Intima/media thickness ratio
  - CNP WT/ApoE KO vs CNP KO/ApoE KO: *

Histological images:
- ecCNP WT/ApoE KO (H & E)
- ecCNP KO/ApoE KO
- α smooth muscle actin

(Brachiocephalic artery)
ecCNP KO phenotyping summary

1. Achieved selective deletion of CNP in endothelial cells

2. Vascular phenotype
   - Loss of endothelium-derived CNP results in impaired resistance artery function *in vitro* and hypertension *in vivo*
   - CNP is involved in the regulation of inflammatory cell recruitment & platelet reactivity (increased endothelial & platelet P-selectin expression)
   - CNP is important for the maintenance of vascular integrity and curbing the progression of atherosclerotic disease

3. Tissue-specific CNP KO is an ideal model to further delineate the importance of CNP in cardiovascular health & disease
   - Assessment of cardiac function in ecCNP KO mice vs. cardiomyocyte (alphaMHC-Cre) CNP KO (ECG telemetry; coronary vascular reactivity; ischaemia reperfusion (I/R) injury; heart failure)

4. Pharmacology infers that the majority of the cytoprotective functions of CNP appear to be mediated via NPR-C
   - Assessing the vascular importance of NPR-B and NPR-C (transgenic approach)
   - Development of small molecule NPR-C agonists
Acknowledgements