Strategies in Cardiovascular Disease and Respiratory Disease

Drug Discovery and Medicinal Chemistry

The Renin-Angiotensin Pathway

Dr Anna Barnard - Spring 2017
Course Overview

Lecture 1:
• Overview of the human heart
• Cardiac agents used to treat
  • Heart failure, Angina and Cardiac arrhythmia

Lecture 2:
• Drugs targeting Adrenergic Receptors (GPCRs)

Lecture 3:
• Agents affecting the Renin-Angiotensin Pathway

Lecture 4:
• Overview of normal lung function
• Drugs for the management of asthma
Learning Objectives

• Explain the reactions carried out in the renin-angiotensin pathway and the biological effects of the products.
• Describe the development of ACE inhibitors and the key interactions they form.
• Describe the peptide mimicry strategy in relation to angiotensin receptor blockers and renin inhibitor development.

Recommended Reading:
The Renin-Angiotensin Pathway

- The renin-angiotensin pathway is a complex, highly regulated pathway integral to controlling blood volume and arterial blood pressure.
- Consists of two main enzymes renin and angiotensin-converting enzyme (ACE).

Historical Overview

- In 1898 Robert Tiegerstedt and his student Pat Bergman demonstrated the existence of a substance in kidney extract which caused an increase in blood pressure.
- This substance was subsequently found to be the enzyme renin and the peptide product was the cause of high BP.
- The peptide, named angiotensin, was found to exist in an inactive (I) and active (II) form.
- Conversion of angiotensin I to angiotensin II is catalysed by a different enzyme – angiotensin converting enzyme (ACE).
Pathway Overview

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-R

Angiotensinogen
Pathway Overview

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-R

Angiotensinogen

Renin

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-R

Angiotensin I
Pathway Overview

Angiotensinogen

Renin

Angiotensin I

Angiotensin Converting Enzyme (ACE)

Angiotensin II

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-R

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile
Pathway Overview

Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Val-Ile-R

**Renin**

Angiotensinogen

Angiotensin I

**Angiotensin Converting Enzyme (ACE)**

Angiotensin II

**Additional Peptidases**

Angiotensin III

Inactive Peptides
Pathway Overview

- Renin is synthesised, stored and secreted from kidney cells.
- These cells are sensitive to the haemodynamic stretch of the blood vessels of the kidney.
- Increased stretch = raised blood pressure = reduced renin release.
- Renin determines the rate of angiotensin II production. ACE is under minimum physiological control and is non-rate limited.
- The only substrate selectivity ACE has is that the penultimate amino acid must not be proline.
- Angiotensin II is a potent vasoconstrictor.
Role in Disease

- The renin-angiotensin pathway is central to maintaining blood volume, pressure and electrolyte balance.
- Pathway overactivation results in hypertension (high blood pressure) or heart failure.
- Lifestyle changes can be sufficient to lower BP.
- As angiotensin II produces the majority of the effects from the pathway, compounds which block either its synthesis or binding can form the basis for effective treatments.
- Drugs include ACE inhibitors, angiotensin II receptor blockers and renin inhibitors.
Angiotensin Converting Enzyme Inhibitors

• ACE inhibitors block the conversion of angiotensin I to angiotensin II.

\[
\text{Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu}
\]

\text{Angiotensin I}

\[
\text{Asp-Arg-Val-Tyr-Ile-His-Pro-Phe}
\]

\text{Angiotensin II}

• There are 3 classes of inhibitors: thiol-containing, dicarboxylate-containing and phosphate-containing.

• Captopril (thiol) and fosinopril (phosphate) are lone representatives of their subclassification.

• ACE inhibitors also inhibit bradykinin degradation.
ACE Inhibitors: Captopril

- In 1965 it was reported that South American pit viper venom potentiated the action of bradykinin.

- Peptide SQ20881 had the greatest potency of the original isolation.
ACE Inhibitors: Captopril

- A hypothetical binding model based on Zn-containing peptidases was proposed.

Zinc is adjacent to the labile peptide bond

Side chains R₁ and R₂ could contribute to overall affinity

Negative C-terminus interacts with positive amine on ACE

C-terminal amide bond forms hydrogen-bonding interactions
ACE Inhibitors: Captopril

- It was noted that succinic acids were potent inhibitors of related peptidase enzymes – fits binding model.
- Given that Proline is the C-terminal amino acid in SQ20881, succinyl-L-proline was synthesised and tested.

- Reasonably selective for ACE but 1/500 as potent as SQ20881.
ACE Inhibitors: Captopril

- Addition of a methyl group to mimic side chain R₂ enhanced activity (1/300 as potent as SQ20881).
- Replacement of the carboxylate with a thiol improved the potency to 200 nM (20x more potent than SQ20881).

- Combining the SAR by addition of a Me resulted in captopril with a $K_i$ of 1.7 nM.
ACE Inhibitors: Enalapril

- The thiol on captopril is also responsible for side effects – skin rashes and taste disturbances.
- Compounds were based on a tripeptide substrate analogue.
- C-terminal proline, Me at $R_3$ and phenylethyl at $R_4$ resulted in enalaprilat.

\[
\text{Enalaprilat} \quad K_i = 0.2 \text{ nM} \\
20x > \text{captopril}
\]

Transition state of angiotensin I
ACE Inhibitors: Enalapril

- Despite excellent activity enalaprilat has poor bioavailability.
- Esterification produced enalapril which is a prodrug activated to enalaprilat by esterases.

\[
\text{Enalaprilat} \quad \text{pKa} = 8 \\
\text{Enalapril} \quad \text{pKa} = 5.5
\]

- Zwitterion formation contributes to low oral activity. Ionisation of the adjacent carboxylate enhances the basicity of the secondary amine.
ACE Inhibitors: Fosinopril

- The search for ACE inhibitors lacking a thiol group led to investigations into phosphorus-containing compounds.
- This phosphinic acid was found to bind to ACE in a similar manner to enalaprilat.
- The phosphinic acid more truly mimics the tetrahedral intermediate.
- The spacing is shorter, unlike enalapril, with the phosphinic acid being only two atoms from the proline. The spacing to the phenyl is overall one atom longer.
ACE Inhibitors: Fosinopril

- Further SAR to investigate more hydrophobic C-terminal ring systems led to fosinoprilat.

- Fosinoprilat is more potent than captopril but less potent than enalaprilat – possibly due to the differences in spacing.

- Fosinoprilat is too hydrophilic to be orally bioavailable so is administered as prodrug fosinopril.
ACE Inhibitors: Fosinopril

- Prodrug fosinopril contains a (acyloxy)alkyl group which is bioactivated by intestinal esterases.
The angiotensin II receptor GPCR was the initial target for drugs to inhibit the renin-angiotensin pathway. Efforts began in the 1970s to develop antagonists based on the natural peptide agonist.
Angiotensin II Receptor Blockers: Losartan

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- Efforts began in the 1970s to develop antagonists based on the natural peptide agonist.

[Chemical structures of Sarcosine, Saralasin, and Losartan are shown, with Sarcosine highlighted in red, Saralasin in green, and Losartan in blue with a red outline.]
Some ‘peptide-mimetics’ were then developed in the 1980s based on angiotensin.
Angiotensin II Receptor Blockers: Losartan

- From S-8038 a number of structural modifications were carried out to improve receptor binding and lipid solubility resulting in losartan with high receptor affinity (19 nM).

![Chemical structures of S-8038 and Losartan]
Angiotensin II Receptor Blockers

- A number of losartan analogues have since been developed.
Angiotensin II Receptor Blockers

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Renin Inhibitors: Aliskiren

- The specificity of renin for its substrate peptide makes it an attractive drug target.
- Initial attempts to develop renin inhibitors focused on the amino acid sequence of angiotensinogen.

- However the cost of preparation of these analogues curtailed commercial interest in this area.
Renin Inhibitors: Aliskiren

- Success was finally obtained when the peptide backbone was abandoned and replaced with a non-peptidic template.
- This template was used in the development of aliskiren, the first non-peptide, low molecular weight, orally active, renin inhibitor.
- Aliskiren has 4 chiral centres and is marketed as the pure 2S,4S,5S,7S-enantiomer.
Summary

• The renin-angiotensin pathway is integral to controlling blood volume and pressure. Pathway overactivation causes hypertension.

• It is controlled by the action of two main enzymes renin and angiotensin-converting enzyme (ACE).

• Drugs have been developed as ACE inhibitors, angiotensin II receptor blockers and renin inhibitors using the native peptides as a model for inhibitor design.