Impaired pressure natriuresis and urinary ET-1 excretion in a rat model of Type I diabetes mellitus

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Renal ET-1 regulates blood pressure through regulation of renal excretion of sodium and water

- Diabetes mellitus causes sodium and water retention which contributes to hypertension
- Hypertension is a major risk factor for diabetic nephropathy
- Endothelin A receptor antagonists can reverse albuminuria in diabetic nephropathy
- Endothelin A receptor antagonists cause sodium and water retention
What is the effect of diabetes mellitus on regulation of renal sodium and water excretion by ET-1?

- To answer this question, we
  - Developed a protocol in which sodium loading could be mimicked, suitable for use in a rat model of diabetes mellitus
  - Measured urinary sodium and water excretion
  - Measured urinary ET-1 excretion

- Comparisons between control rats and a Type I diabetic rat model
Sodium loading can be mimicked by increasing renal perfusion pressure

![Graph showing the relationship between urine flow rate, urinary sodium excretion, mean arterial blood pressure, and renal perfusion pressure.](image-url)
Hypotheses

• Urinary sodium and water excretion after sodium loading is impaired in the diabetic state

• Impaired urinary sodium and water excretion in diabetes mellitus is associated with reduced renal ET-1 excretion
Aims

1. Can we adapt a protocol of pressure diuresis/natriuresis to suit a diabetic rat model?

2. Using this protocol in a diabetic rat model:
   a) Is pressure diuresis/natriuresis impaired?
   b) Is urinary ET-1 excretion impaired?
   c) Is the relationship between urinary ET-1 and urinary sodium and water excretion impaired?
Pressure diuresis/natriuresis protocol

- Adapted from Roman and Cowley (1985)
- Similar to Schneider et al (2008)

- 15 adult male Sprague Dawley rats
  - 8 control
  - 7 diabetic

- Left jugular vein
  - Inulin (glomerular filtration rate)

- Left carotid artery
  - Direct blood pressure
  - Blood sampling

- Tube cystotomy

- Pre-placed ligatures
  - Coeliac artery
  - Cranial mesenteric artery
  - Distal aorta- Ligation 2

Ligation 1
Timeline

<table>
<thead>
<tr>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>-110 mins</td>
<td>START</td>
</tr>
<tr>
<td>0 mins</td>
<td>URINE BASELINE</td>
</tr>
<tr>
<td>30 mins</td>
<td>URINE CLEARANCE 1</td>
</tr>
<tr>
<td>60 mins</td>
<td>URINE CLEARANCE 2</td>
</tr>
<tr>
<td>90 mins</td>
<td>FINISH EUTHANASIA</td>
</tr>
</tbody>
</table>

LIGATION 1: Coeliac artery
LIGATION 2: Distal aorta
Cranial mesenteric artery
Diabetic rat model

- Streptozotocin-induced model of Type I diabetes mellitus (30mg/kg ip)
- Diabetic status confirmed after 2 days
- Diabetic rats underwent pressure diuresis/natriuresis 14-21 days post induction
Ligation of major arteries increased arterial blood pressure in a stepwise manner in both control and diabetic rats.

Mean arterial blood pressure P = 0.005

- Control
- Diabetic
Elevated mean arterial blood pressure increased urine flow rate and urinary sodium and ET-1 excretion rates but far less in diabetic rats than in control rats.

**Mean arterial blood pressure** $P=0.005$

- Control
- Diabetic

**Urine flow rate** $P=0.001$

**Urinary sodium excretion rate** $P=0.011$

**Urinary ET-1 excretion rate** $P=0.004$
Diabetes mellitus impairs pressure diuresis

**Urine flow rate control**

\[ R^2 = 0.42 \]

\[ P < 0.001 \]

**Urine flow rate diabetic**

\[ R^2 = 0.36 \]

\[ P = 0.004 \]
Diabetes mellitus impairs pressure natriuresis.

* Urinary sodium excretion rate control
  - $R^2=0.51$
  - $P<0.001$

* Log UNaV diabetic
  - $R^2=0.21$
  - $P=0.087$

Control

Diabetic
Differences in urine flow rate and urinary sodium excretion rate reflect inappropriate sodium resorption rather than reduced filtered sodium load.
In diabetes mellitus, the relationship between urine flow and urinary ET-1 excretion is unchanged.

R=0.69  
P=0.01

R=0.85  
P<0.001

Control

Diabetic
In diabetes mellitus, the relationship between urinary sodium and ET-1 excretion is unchanged.

- Control: $R=0.51$, $P<0.001$
- Diabetic: $R=0.61$, $P=0.017$
Summary

In this model

• Diabetes mellitus profoundly impairs pressure diuresis and natriuresis

• Urinary ET-1 excretion rate is also reduced

• Urinary ET-1 excretion rate has a strong positive correlation with:
  ▪ Urine flow rate
  ▪ Urinary sodium excretion rate

Regardless of diabetic status
Discussion

1. Can we adapt a protocol of pressure diuresis/natriuresis to suit a diabetic rat model?
   Yes

2. Using this protocol in a diabetic rat model:
   a) Is pressure diuresis/natriuresis impaired?
      Yes
   
   b) Is urinary ET-1 excretion impaired?
      Yes
   
   c) Is the relationship between urinary ET-1 and urinary sodium and water excretion impaired?
      No, there remains a strong relationship between urine flow and urinary sodium and ET-1 excretion

• This could explain the sodium and water retention observed in diabetes mellitus

• Impaired pressure natriuresis is associated with impaired urinary ET-1 excretion
We now wish to investigate the causal nature of the relationship between impaired urinary ET-1 excretion and impaired urinary sodium and water excretion in diabetes mellitus.

• What effect does manipulation of renal endothelin signalling have on pressure diuresis/natriuresis?
  - Selective ETA and ETB receptor antagonism

• How is this effect altered in the diabetic state?
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