(--)-Englerin A as a novel potent activator of TRPC4 and TRPC5 channels

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Introduction to TRP channels

- Non-selective cation channels
- Ubiquitously expressed throughout the human body
- 7 families of TRP channels
  - C = ‘canonical’
- 6 membrane spanning domains with the pore loop between S5 and S6
- Often expressed as heteromeric channels
- Lack of specific small molecule modulators
TRP channel activation by natural chemicals – TRPC?

capsaicin
heat

menthol
cold

wasabi
mustard oil
cinnamon

TRPV1

TRPM8

TRPA1

TRPC-

Ca\(^{2+}/Na^+\)

Ca\(^{2+}/Na^+\)

Ca\(^{2+}/Na^+\)

Ca\(^{2+}/Na^+\)
Inhibition of TRPC5 by an omega-3 fatty acid

α-Linolenic acid (ALA) is an essential omega-3 fatty acid found in seeds, nuts and many common vegetable oils.
Inhibition of TRPC5 by a chemical extract from ginger

A traditional Chinese medicine for cardiovascular complaints

Naylor et al - In preparation
The discovery of (-)-Englerin A

(-)-Englerin A is an extract from *Phyllanthus engleri*

Heinrich Engler (1844-1930)
*German botanist*
Englerin A, a Selective Inhibitor of Renal Cancer Cell Growth, from *Phyllanthus engleri*

**Natural Product Synthesis**

Total Synthesis and Absolute Configuration of the Guaiane Sesquiterpene Englerin A**

Matthieu Willot, Lea Radtke, Daniel Könning, Roland Fröhlich, Viktoria H. Gessner, Carsten Strohmann, and Mathias Christmann*
Renal cell carcinoma

61,560 new cases in USA (2015)

The most common type of kidney cancer

7th most common cancer in UK

Resistant to chemotherapy

Often asymptomatic until it spreads to other organs
The ‘Englerin team’
Synthesis and target identification

A498 = Renal cell carcinoma cell line

(-)-Englerin A

Akbulut Gaunt et al 2015 Angewandte Chemie Int Ed
Potent stereo-selective activator of TRPC4

(-)-Englerin A evokes calcium entry in HEK cells overexpressing TRPC4
Extremely efficacious activator

HEK C4

100 nM (-)EA

20 μM La³⁺

+100 mV

-100 mV

Time (min)

I (nA)
Activates TRPC4 and TRPC5 with nanomolar potency

**HEK-TRPC4**

- EC$_{50}$ 11.2 nM

**HEK-TRPC5**

- EC$_{50}$ 7.6 nM

Activates TRPC4 and TRPC5 with nanomolar potency.
Specific TRPC4/5 channel activator

- No effect on 14 other ion channels or 59 GPCRs
- No binders detected in unbiased proteomic screening
Powerful activation independent of G-proteins

Outside-out patch configuration

ML204
TRPC4/5 channel inhibitor
TRPC4 IC$_{50}$ = 0.99 μM
TRPC5 IC$_{50}$ = 9.2 μM

HEK-TRPC4

(-)EA (nM)
100 100 100 1000 100 100

ML 5 μM

[Graph showing current (I) vs. time (min) for HEK-TRPC4 with different concentrations of (-)EA and ML 5 μM]

HEK C4

(-)EA-evoked
I (nA)

V (mV)

[Graph showing voltage (V) vs. current (I) for HEK C4 with (-)EA-evoked response]
Extracellular effect

Outside-out patch configuration
(-)-Englerin A present in the intracellular pipette solution throughout
(-)-Englerin A acts potently to evoke calcium entry in A498 cells

**Graphs:**
- **Left:** Graph showing calcium entry over time with (-)-EA or vehicle.
- **Right:** Graph showing dose-response relationship with (-)-EA, showing EC50 at 9.3 nM.
- **Bottom Left:** Bar graph comparing vehicle, (-)-EA, and (+)-EA.
- **Bottom Right:** Bar graph comparing (-)-EA, (+)-EA, and ML204.

**Legend:**
- A498
- (-)-EA or vehicle
- (-)-EA
- vehicle
- ML204
- (-)-EA
- (+)-EA
- ns
- *
The native channels act remarkably like heteromers
TRPC1 and TRPC4 are involved in the mechanism of cell death

Blocking antibodies

siRNA

Hannah Gaunt

Melanie Ludlow
Emerging correlation
TRPC1/4 expression, cell death and ionic current

Cell death

Protein

Ionic current

HT-29 colorectal cancer cell line

Melanie Ludlow, Hannah Gaunt, Katsuhiko Muraki
A498 cells were subcutaneously implanted on the left flanks of 8 week old SCID beige mice.

- 2 mg / kg (-)-EA or DMSO treatment (n=4 each)

- Intraperitoneal injection daily
Summary

- (-)-Englerin A **selectively** activates TRPC4 and TRPC5 channels with **high potency and efficacy**

- Acts on the **extracellular** side of the membrane independent of G-protein activation

- TRPC1/4 protein expression **correlates** with the susceptibility to (-)-Englerin A evoked cell death

- (-)-Englerin A activated **TRPC1/4 channels** are involved in its mechanism of cell death

**Implications:**
1. A potential route to a renal cell carcinoma therapy
2. A channel activator in screens for TRPC4/5 inhibitors
3. For understanding TRPC4/5 channel biology
Thank you!

**The University of Leeds, UK**
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Wellcome trust

British Heart Foundation

Cardiovascular Research Leeds UK
Out of Africa: a natural born killer

TRPC4 activators as selective chemotherapy "magic bullets" for renal cell carcinoma!?