"An Old Argument with a New Paradigm"

2-Methoxyestradiol a Specific Pharmacological Inhibitor for the Angiotensin Type 1 Receptor and Hypertension

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Observation

The study shows 2ME2 reduces blood pressure possibly by AT1R down-regulation through a G-protein coupled receptor (GPR30) tethering with epidermal growth factor receptor (EGFR) induced MAP-Kinase pathway.

Hypertension Facts

According to the AHA estimates:

- About 76.4 million people in the United States age 20 and older have high blood pressure.
- Nearly one in three U.S. adults has hypertension, but because there are no symptoms, nearly one-third of these people don't know they have it.

Hypertension is called the "silent killer."

You can't feel it or see it, however

"you cannot afford to ignore it"

Health Consequences of Hypertension Men Vs. Women with Hypertension

Compare a 50 year-old individual of normal body mass with normal blood pressure (120/80) to high blood pressure (146/86) has:

Diseases	Risk of Dying	
	For Men	For Women
Heart attack	3X	4X
Heart failure	2X	3X
Stroke	4X	4X
Kidney Disease	3X	3X

Cardioprotective Effects of Estrogen

Premenopausal women have shown reduced risk for cardiovascular diseases compared to men of similar age

- **Increases insulin sensitivity in resistant individuals**
- **Inhibits cholesterol deposition and LDL oxidation**
- **Increases HDL levels**
- **Anti-oxidant properties**

Gouva L. and Tsatsoulis A. The role of estrogens in cardiovascular disease in the aftermath of clinical trials. Hormones. 2004 Jul-Sep;3(3):171-83.

Adverse Effects of Estrogen

Heart and Estrogen/progestin Replacement Study(HERS)

- **Risk of coronary heart disease(CHD)**
  - **Atherosclerosis**
  - **Heart Attack**
  - **Cardiac Arrest**
- **Increased risk of stroke and blood clots**
- **Increases breast cancer incidence, lung and uterine cancer**
- **Thromboembolsim and gall bladder disease**

- *Women's Health Initiative-NCI Cancer Bulletin, April 2011 edition*
- *Manson JE, Hsia J,et al. N Engl J Med. 2003 Aug 7;349(6):523-34*
- *Hulley S, Grady D, Bush T , et al. JAMA. 1998;280:605-613.*

Blood Pressure Reduction

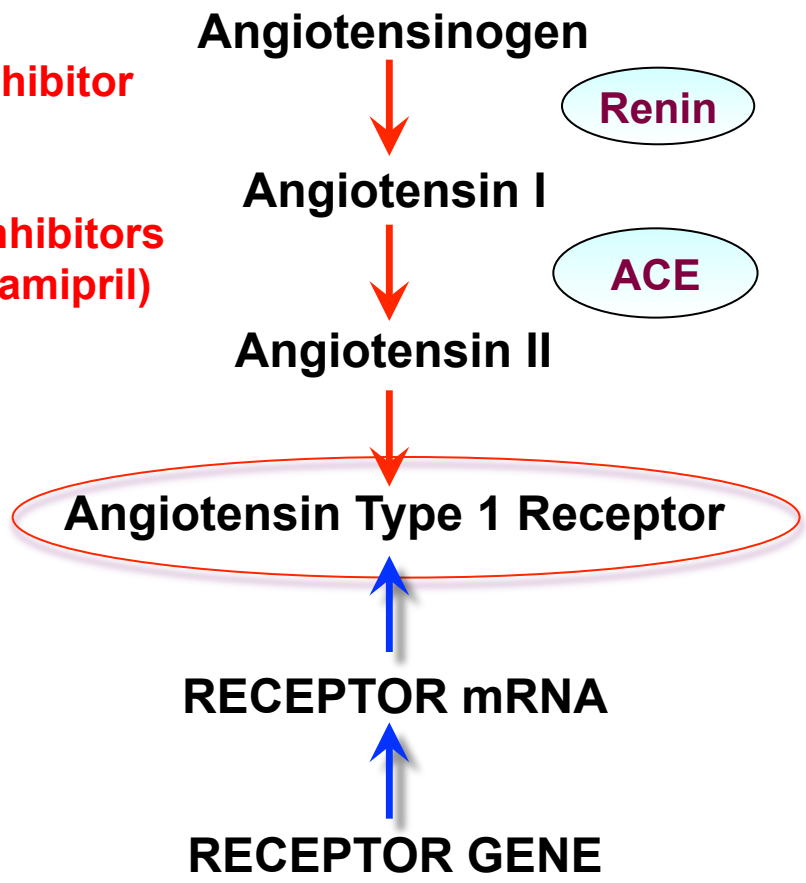
- Dietary and lifestyle changes
  - Eat more fruits, vegetables & low-fat dairy products
  - Physical Activity, lose weight, drink less alcohol
- Antihypertensive drug therapy
  - Vasodilators, Sympathetic nerve inhibitors, ACE inhibitors, Angiotensin Receptor blockers, Diuretics, Calcium channel blockers

Control of AngII Actions *(Drug Targets)*

"Current Drug" Renin inhibitor (Aliskiren)

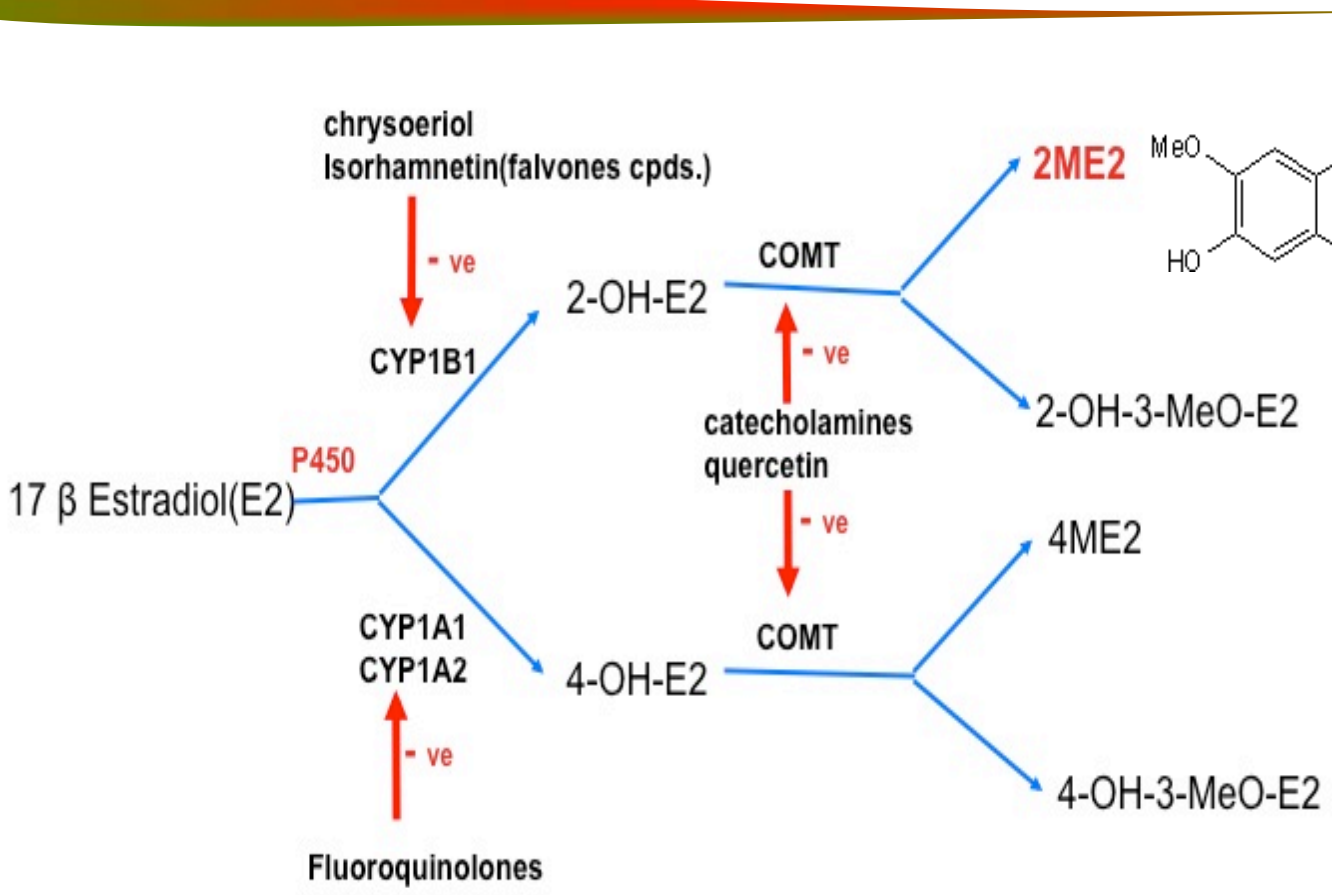
"Current Drugs" ACE inhibitors (Captopril, Enalapril, Ramipril)

"Current Drugs" ARBs (Candesartan, Losartan, Valsartan)



Disease Target

Metabolism of Estradiol to 2-Methoxyestradiol

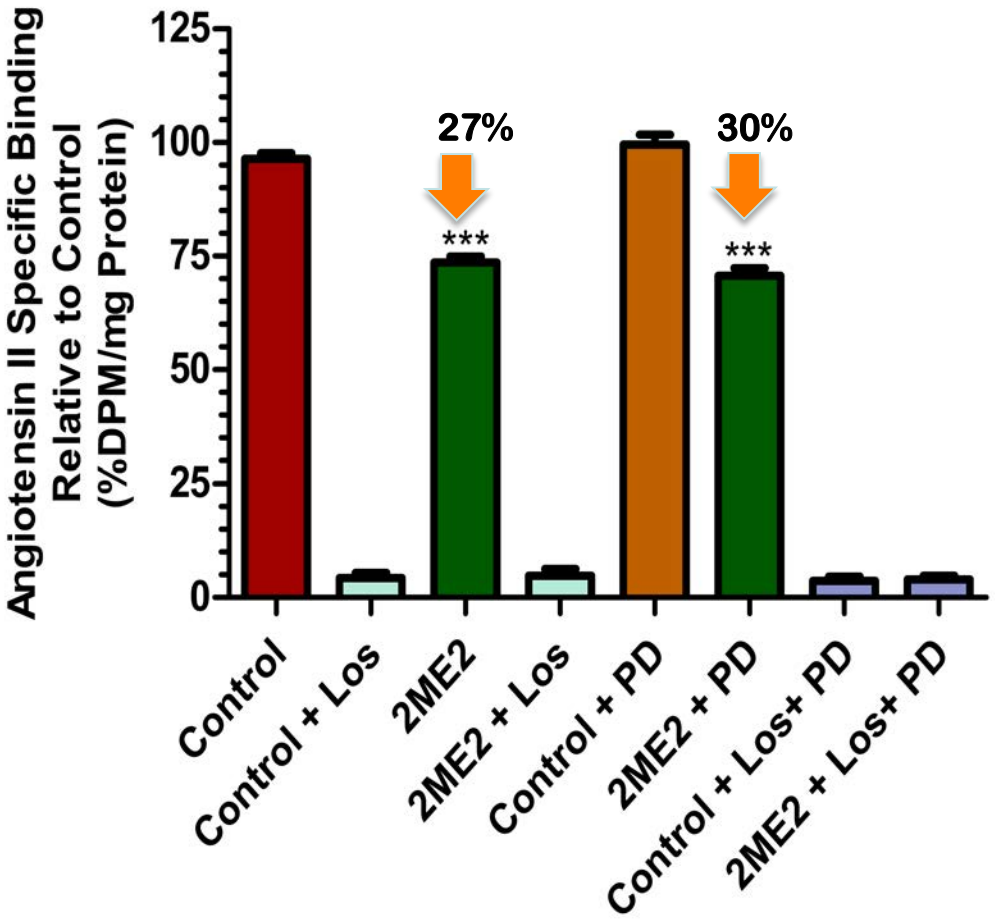


Methoxyestradiols Mediate the Antimitogenic Effects of Estradiol on Vascular Smooth Muscle Cells Via Estrogen Receptor-Independent Mechanisms. Dubey, R. K., D. G. Gillespie, L. C. et al.. Biochem Biophys Res Commun 278, 27-33, 2000

Hypothesis

Cardioprotective effects of estrogen are primarily due to its metabolite 2-Methoxyestradiol (2ME2) mediated down-regulation of angiotensin type 1 receptor (AT1R) expression.

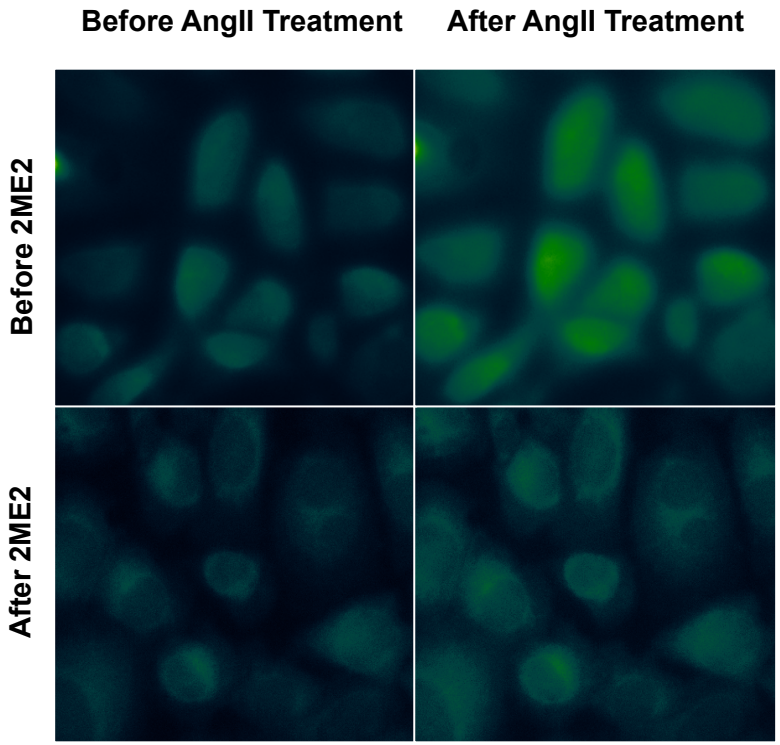
2ME2 Inhibits AT1R Specific Binding



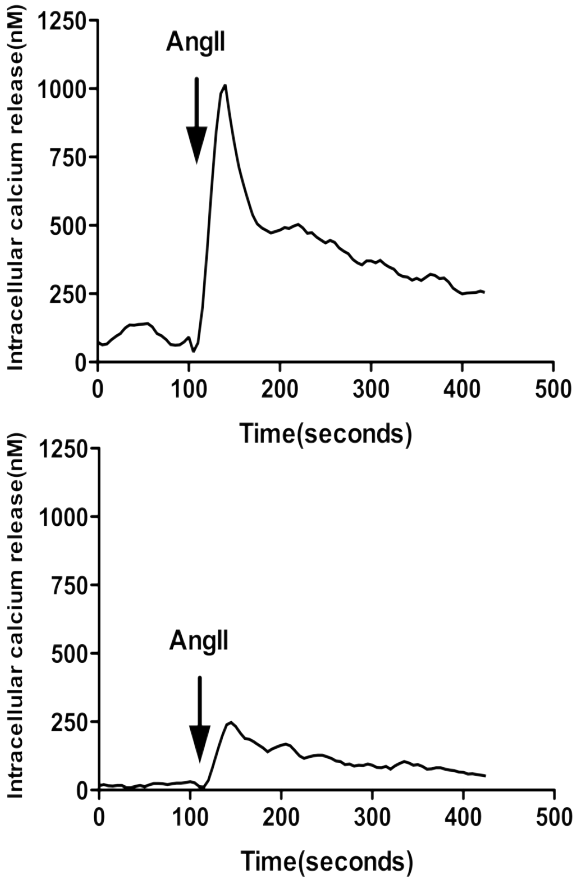
Data are expressed as mean ± SEM (N=3). ***P<0.0001 versus untreated control.

Koganti, et al. Gender Medicine, 2012

2ME2 Significantly Inhibited AngII Mediated Increase in Intracellular Calcium



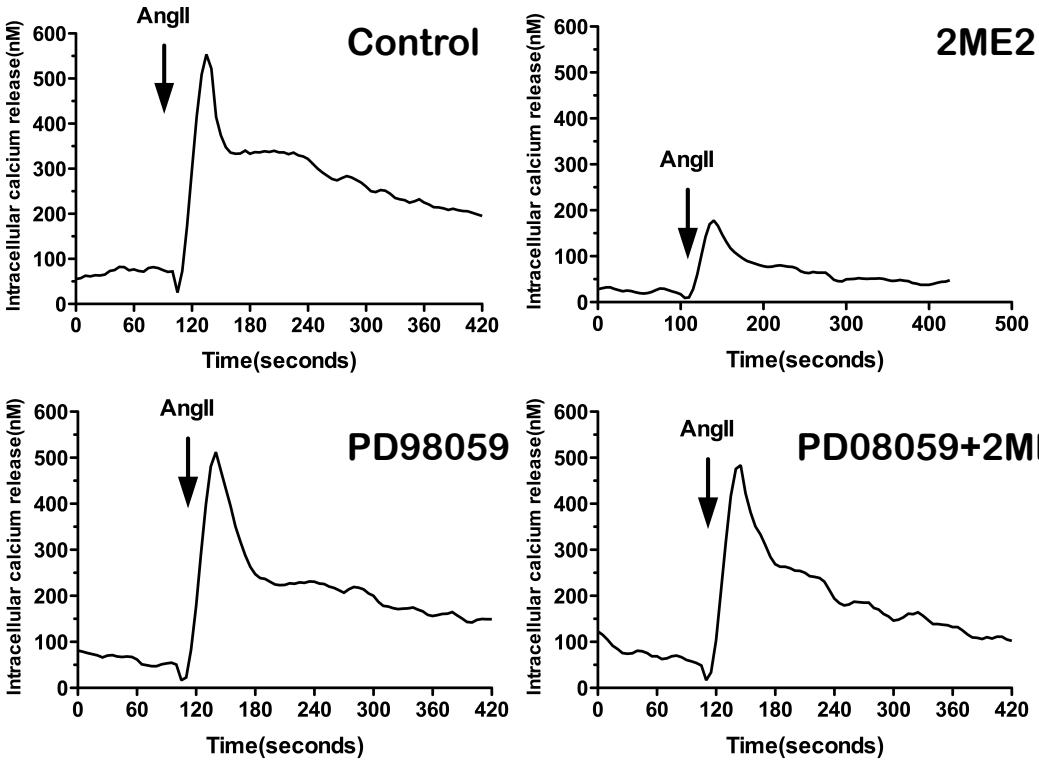
Representative images, N=3

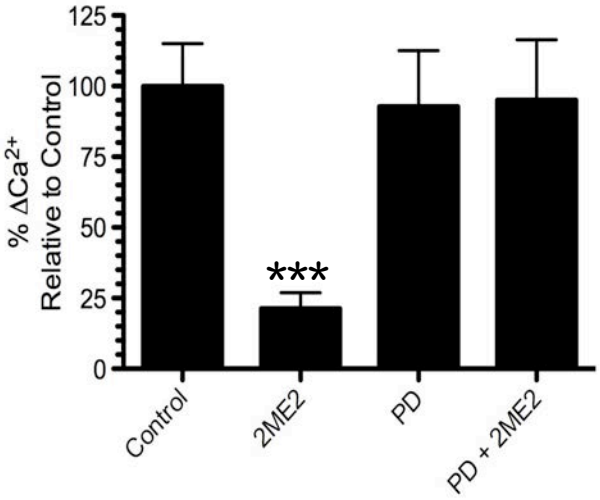


Representative tracings, N=3

Koganti, et al. Gender Medicine, 2012

2ME2 inhibition of AngII mediated increase in Intracellular Calcium is MAP Kinase mediated

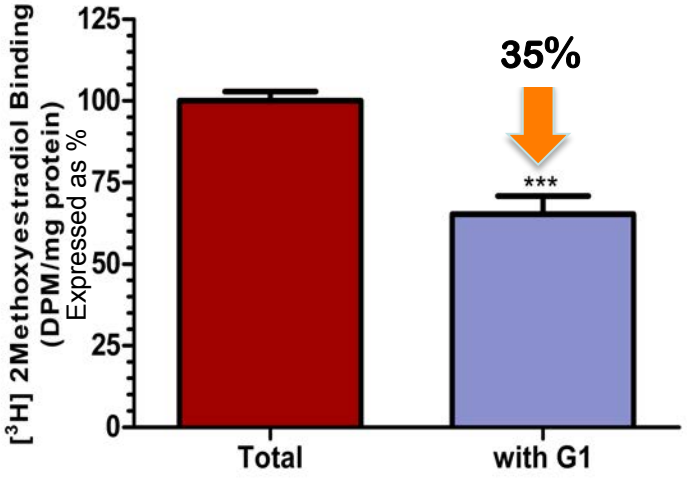




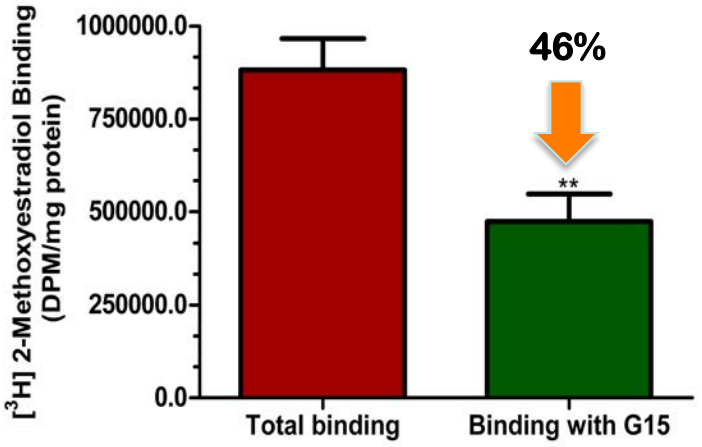
Data are expressed as mean±SEM (N=3). ***P<0.001 compared to control.

Koganti, et al. Gender Medicine, 2012

GPR30 Agonist G1 or Antagonist G15 Displaces [^{3}H]2ME2 Specific Binding



Data are expressed as mean±SEM (N=3).
****P=0.0003 versus total binding.*

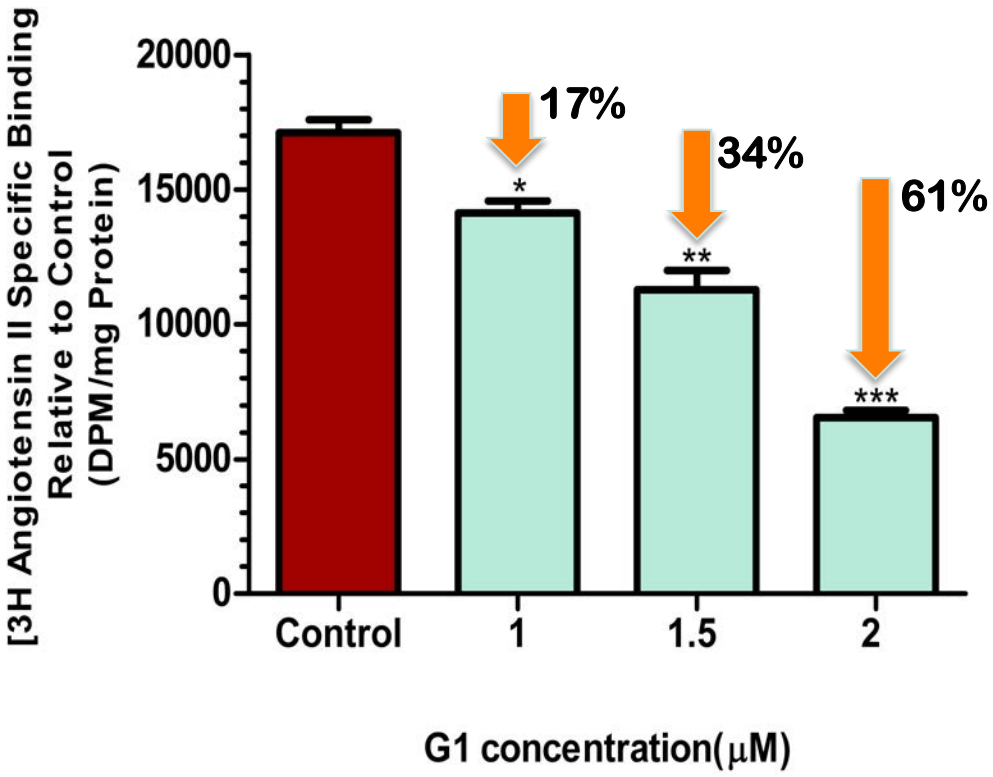


Data are expressed as mean±SEM (N=3).
***P=0.002 versus total binding.*

G1 Formula $C_{21}H_{18}BrNO_3$
G15 Formula $C_{19}H_{16}BrNO_2$

Koganti, et al. Eur J Pharmacol. 2014

GPR30 Agonist G1 Down-regulates AT1R Specific Binding Independent of 2ME2

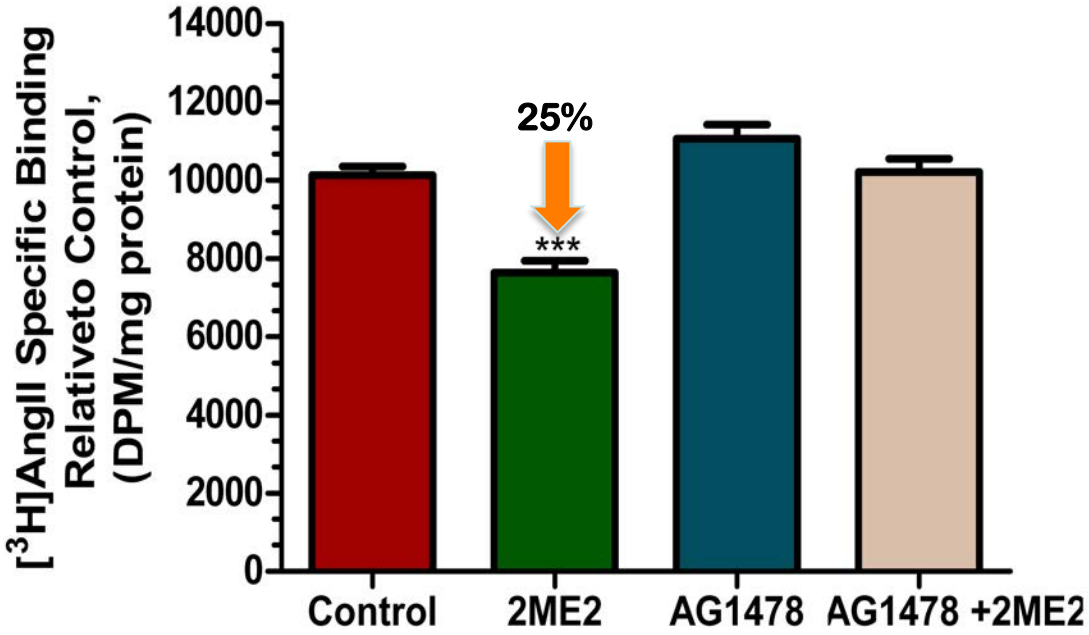


Data are expressed as mean ± SEM (N=3). ***p<0.0001 versus total untreated control.

Koganti, et al. Eur J Pharmacol. 2014

EGFR inhibitor Reverses 2ME2 Response

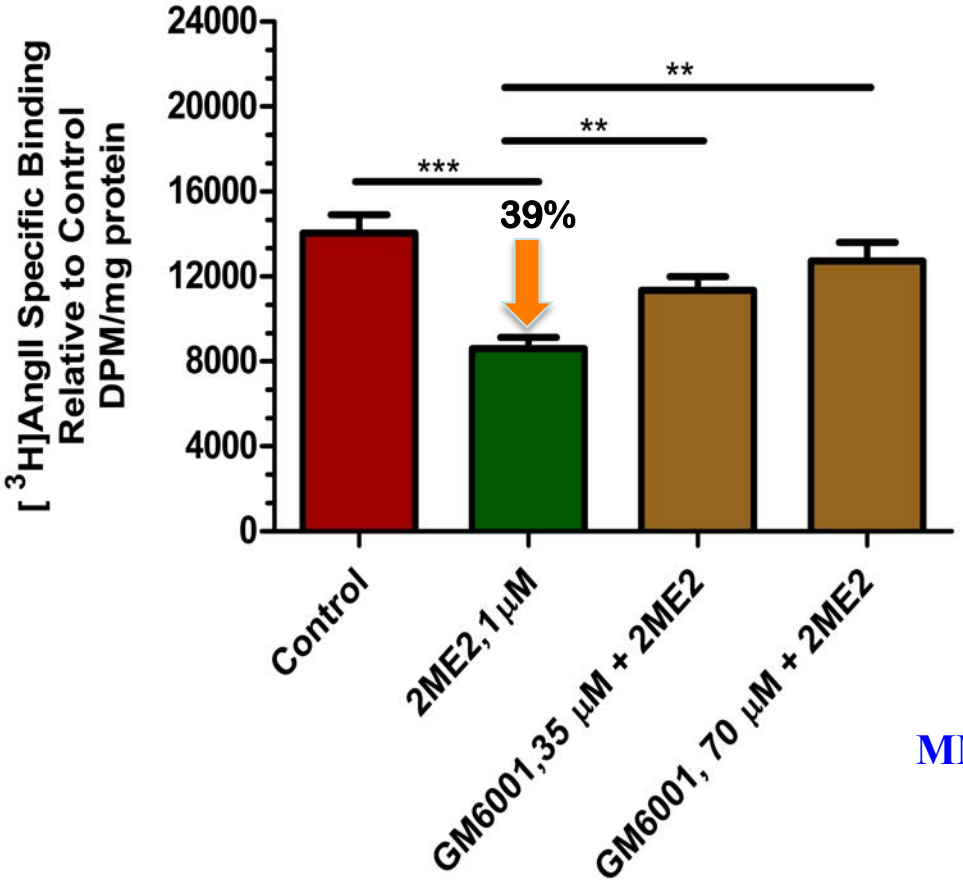
2ME2 = 1μM
EGF Inhibitor AG1478 = 10nM



Data are expressed as mean± SEM (N=3). P<0.001

Koganti, et al. Eur J Pharmacol. 2014

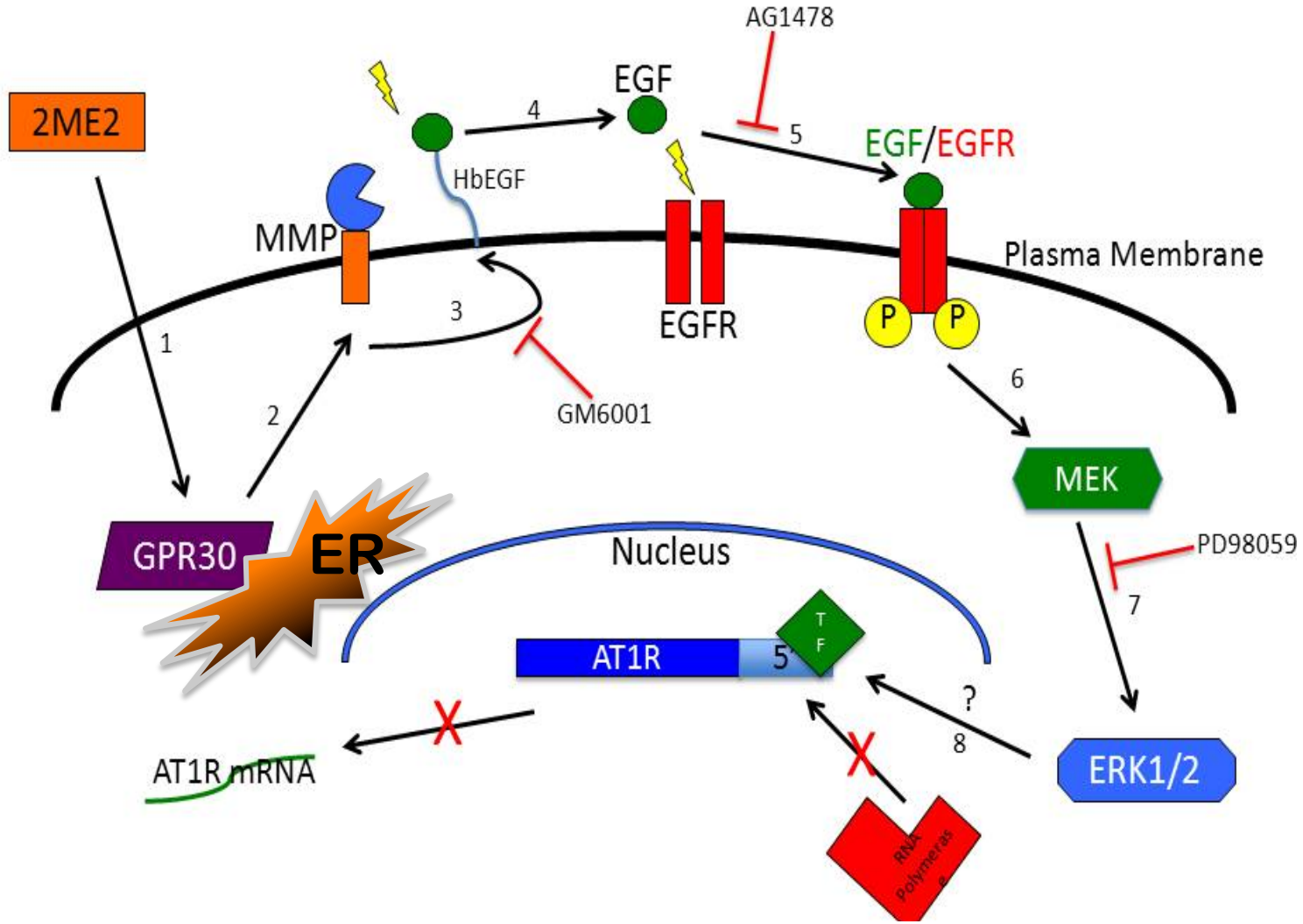
2ME2 mediated inhibition of AT1R expression is Matrix Metalloproteinase Dependent



MMP inhibitor-GM6001

Data are expressed as mean± SEM (N=3). ***P=0.001, **P=0.6423

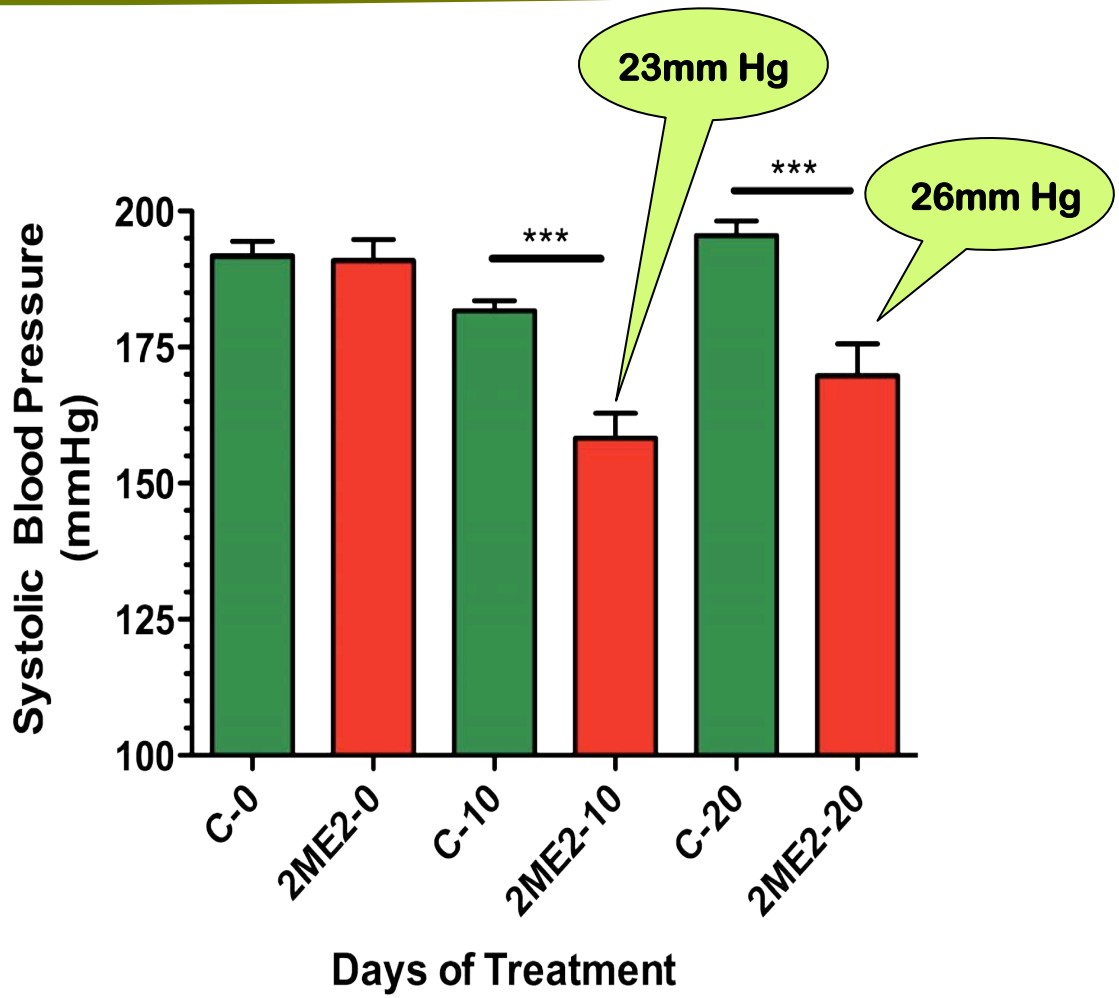
Proposed Mechanism(s) of 2ME2 Actions



Animal Study (*in vivo* validation)

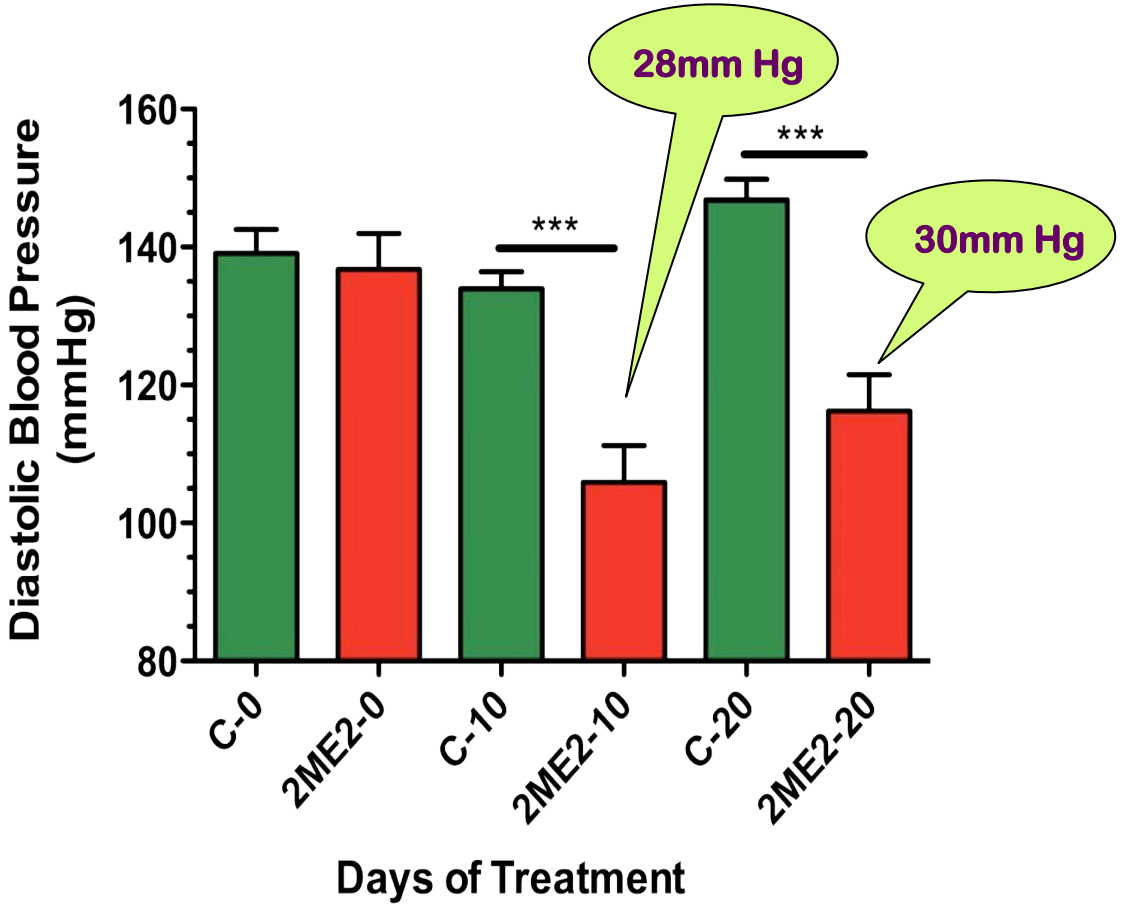
- Spontaneously Hypertensive Rats
- Male, 14 week, 290-320 grams
- 12 hour day/night cycle
- Weight taken every other day
- 10mg/kg 2ME2 delivered IP (every day in the evening)
- Study performed for 3 weeks
- Blood Pressure measured (every other day in the morning)
- At the end of the study renal cortex isolated for analysis

2ME2 Reduces Systolic Blood Pressure in SHR



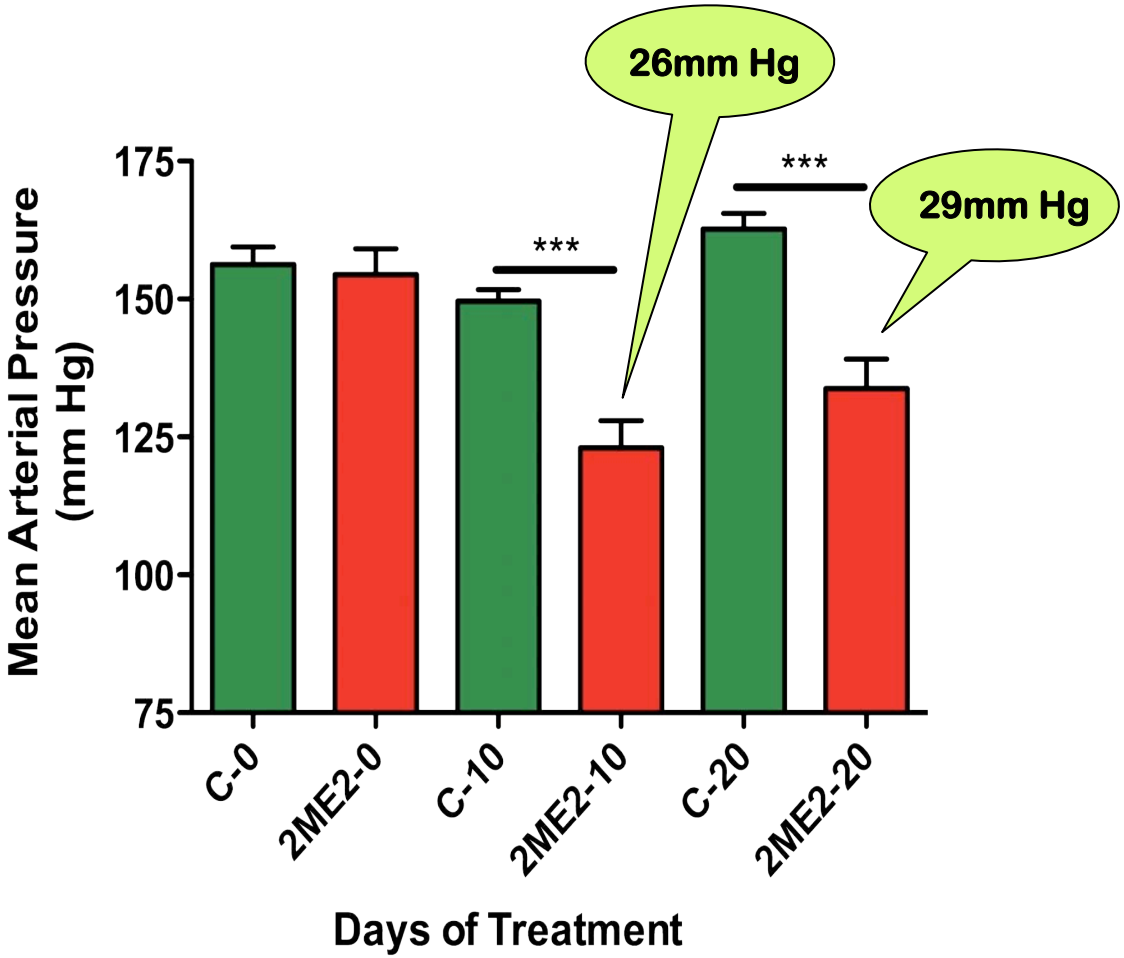
Data are expressed as mean ± SEM (N=4). *** P<0.0001.

2ME2 Reduces Diastolic Blood Pressure in SHR



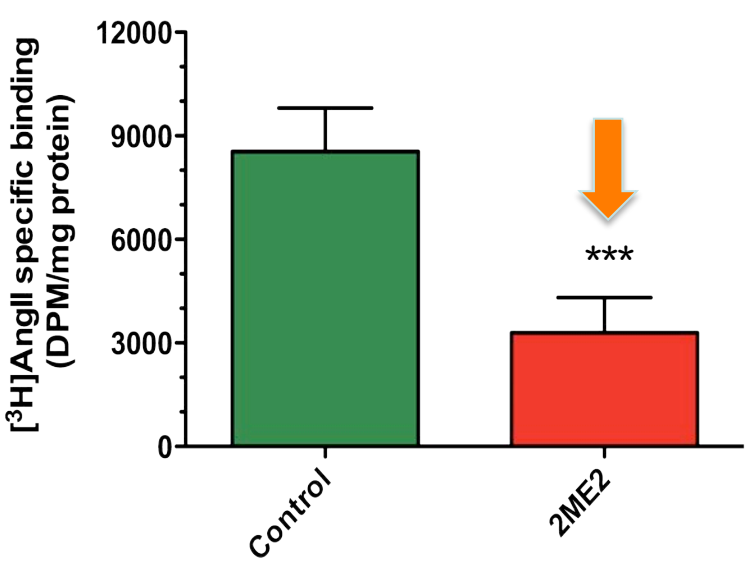
Data are expressed as mean ± SEM (N=4). *** P<0.0001.

2ME2 Reduces Mean Arterial Pressure in SHR

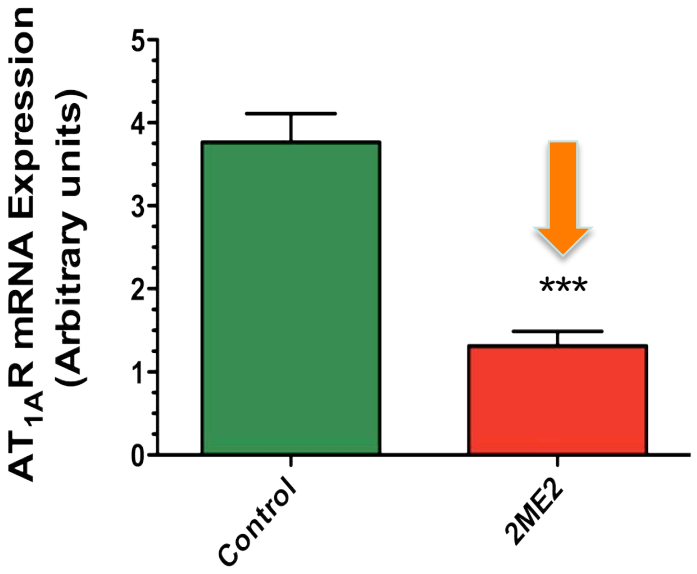


Data are expressed as mean ± SEM (N=4). *** P<0.0001.

2ME2 Treatment Down-Regulates AT1R Protein and mRNA in Renal Cortex of SHR

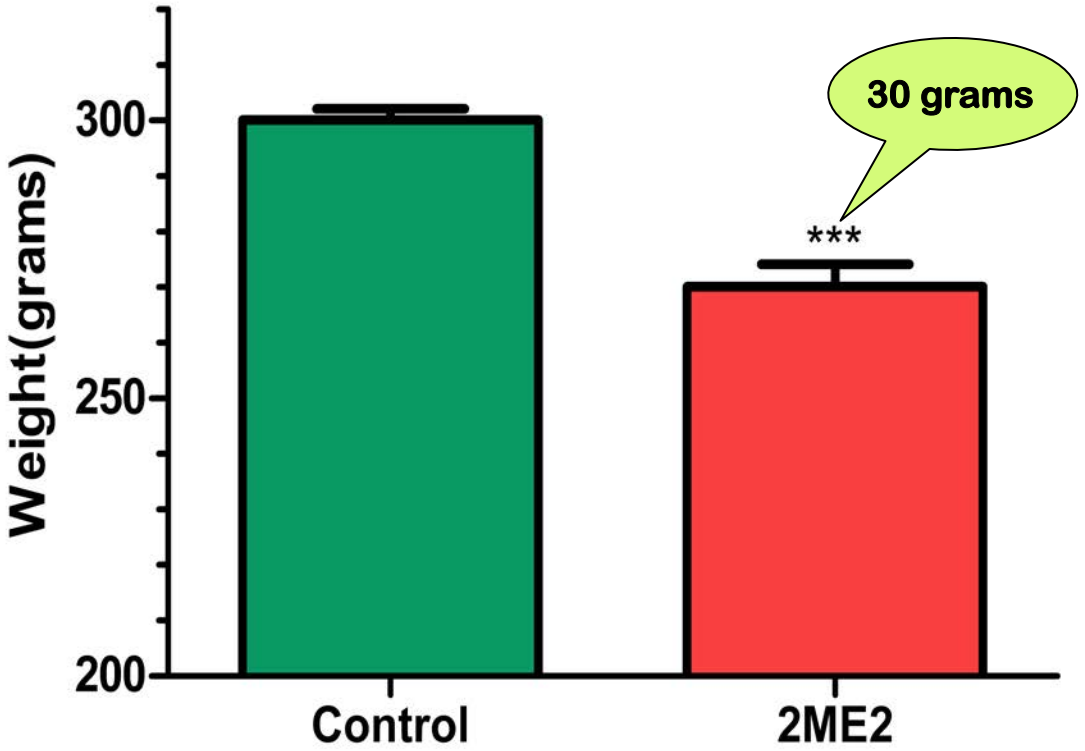


Data are expressed as mean ± SEM (N=4).
*** P<0.0001 versus control group.



Data are expressed as mean ± SEM (N=4).
*** P=0.0007 versus control group.

2ME2 Reduces Body Weight of SHR



Data are expressed as mean ± SEM (N=4). ***P<0.0001 versus control group.

Summary

1. **In Cells 2ME2 down-regulates AT1R expression in a dose and time dependent manner**
2. **AT1R down-regulation lead to decrease in AngII mediated release in intracellular Calcium levels**
3. **2ME2 mediated down-regulation of AT1R is GPR30 and MAP-Kinase(ERK 1/2) dependent**
4. **GRP30 induced down-regulation of AT1R is MMP and EGFR dependent**
5. **2ME2 significantly reduced the blood pressure in SHR**

2ME2 mediated changes in AT1R expression may provide beneficial effects to cardiovascular disorders such as hypertension

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