

Studio degli effetti *in vitro* del *Paracetamolo* sul sistema GnRH

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Master Degree in Safety Assessment of Xenobiotics and Biotechnological Products

Progetto svolto presso Department of Endocrinology The William Harvey Research Institute

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Paracetamolo



- Utilizzato in gravidanza e nell'infanzia
- Riesce ad infiltrare nella BBB e la placenta
- Analgesico ed antipiretico



Home > Advertising Investigations: September 2016

Medicines & Healthcare
products
Regulatory Agency

Decision September 2016: Multiple sales of analgesic-containing in retail outlets

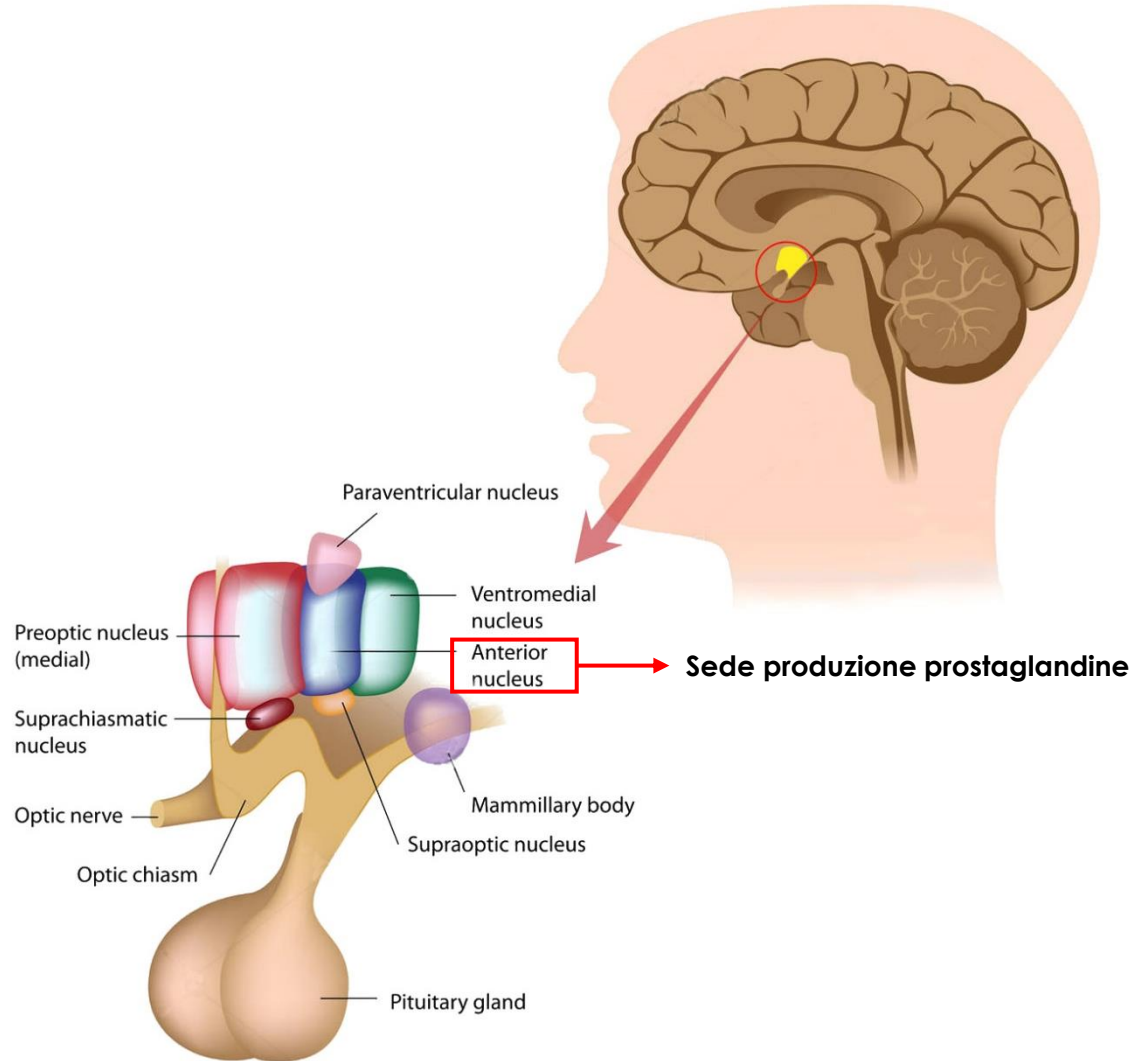
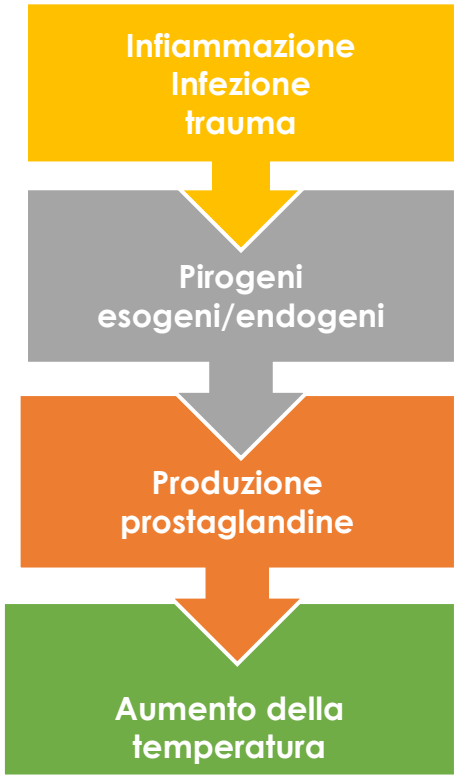
Published 19 October 2016

A [BMJ](#) article - "How well are national guidelines relating to the general sales of aspirin and paracetamol, adhered to by retail stores: a mystery shopper study" - of 18 January 2016, and a [Chemist and Druggist \(C+D\)](#) article - "The paracetamol challenge" - of 27 November 2015 that provided evidence that some retailers were not adhering to the MHRA [Best practice guidance](#) on the sale of medicines for pain relief, were brought to our attention. In addition some of the sales exceeded the legal limit of 100 tablets of paracetamol or aspirin per transaction. Above this level, the supply becomes subject to

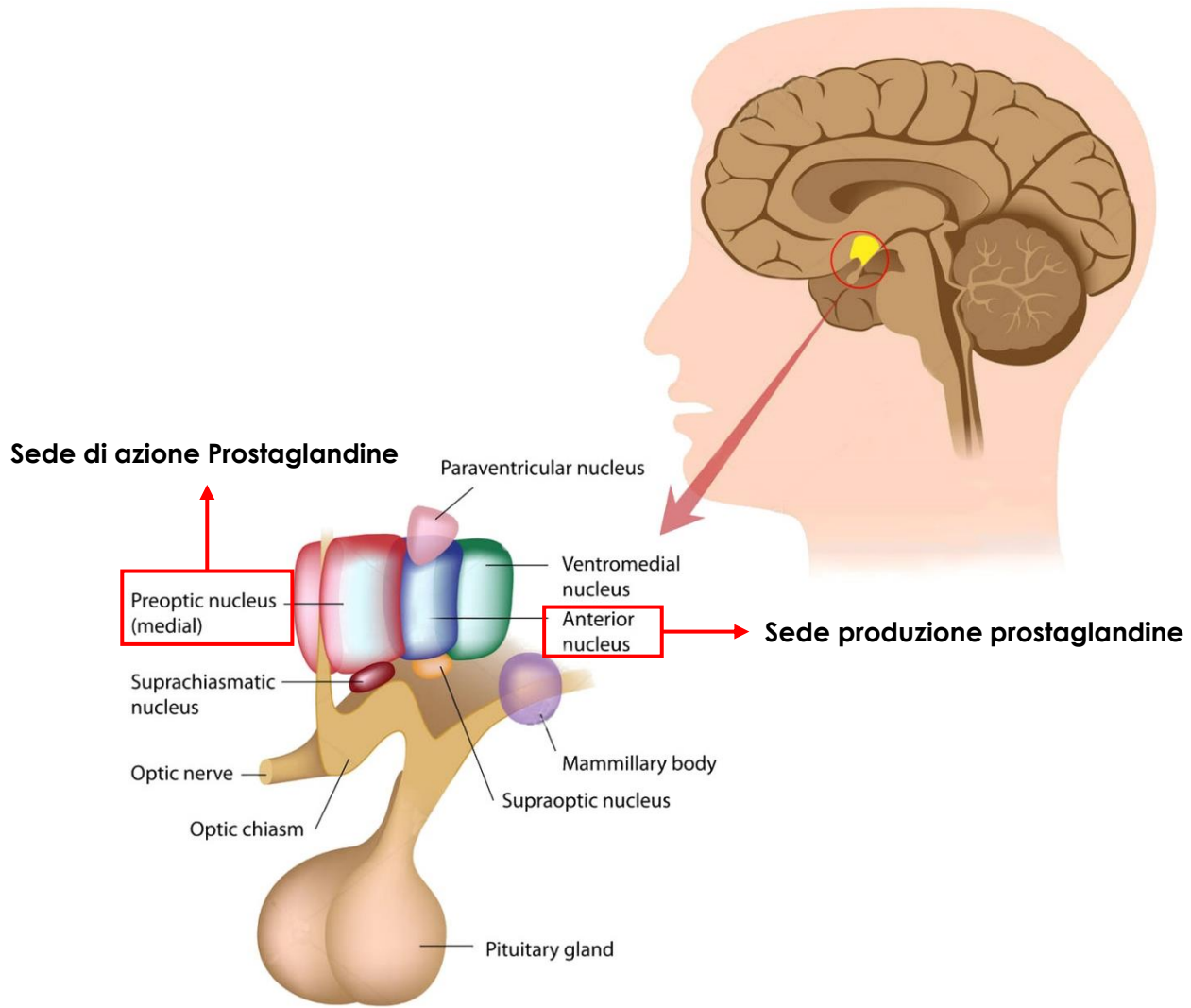
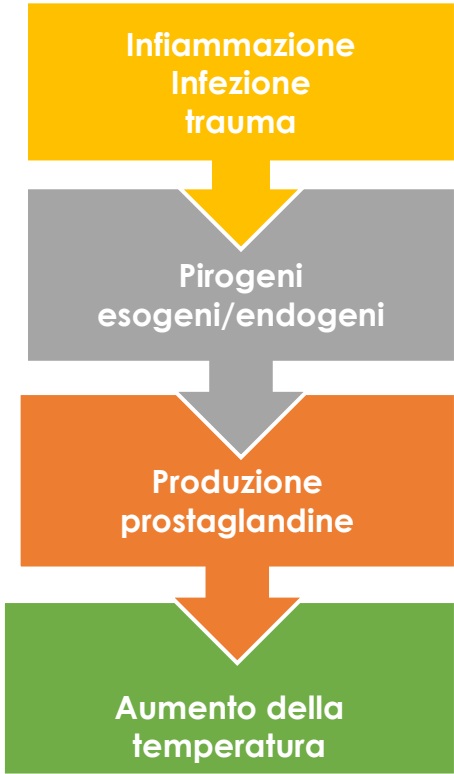


- Economico
- Ben tollerato
- Rari effetti indesiderati
- Bassa tossicità nei bambini
- Biodisponibilità
- Eliminato velocemente

Il Paracetamolo agisce sull'Ipotalamo

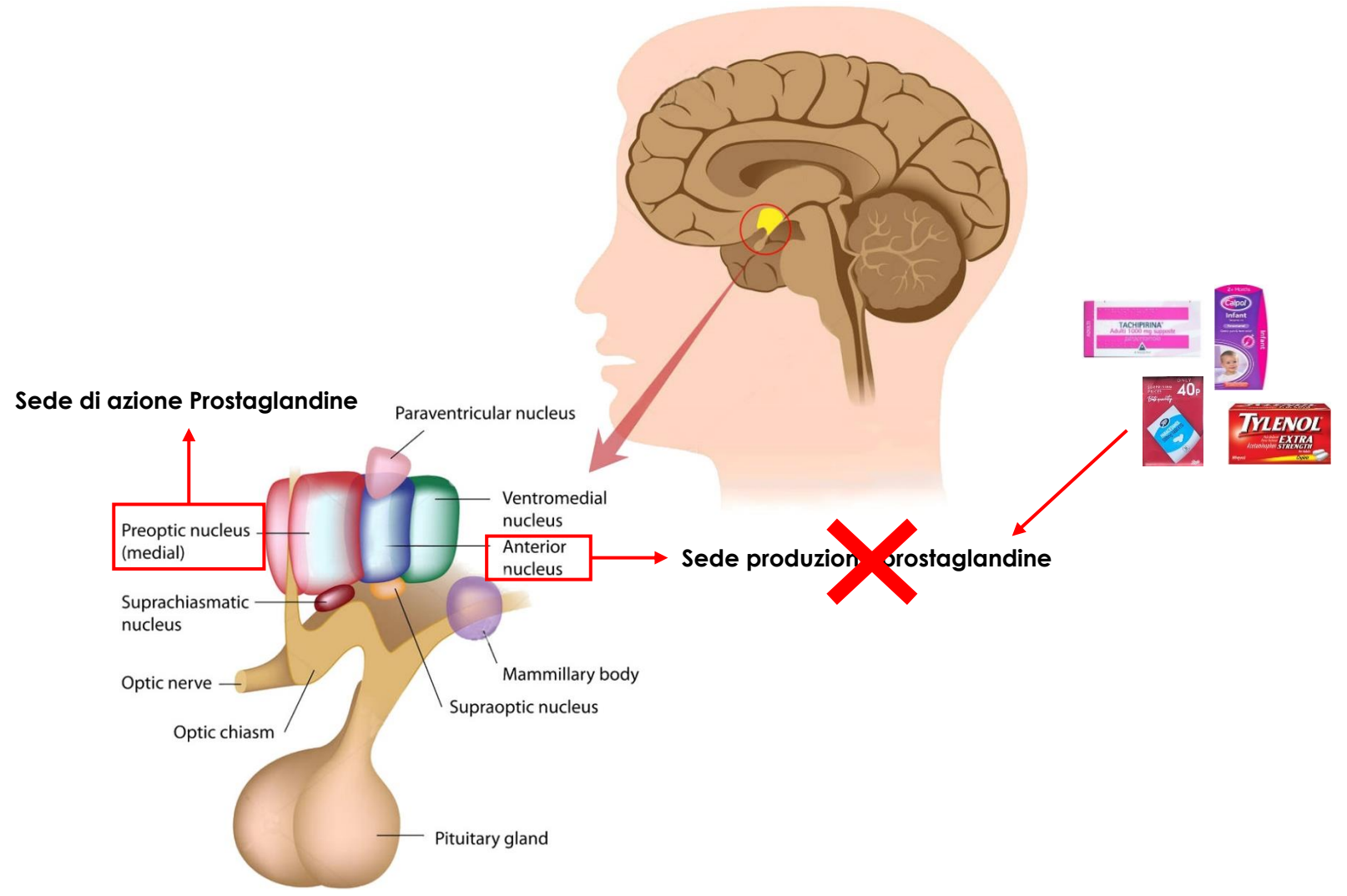
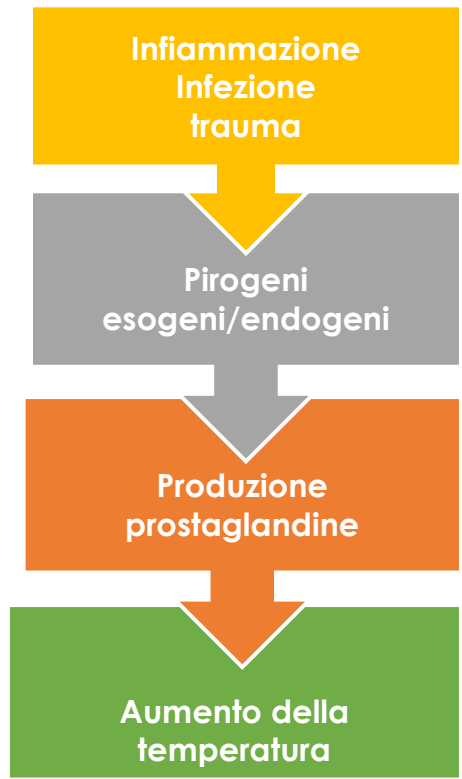


Il Paracetamolo agisce sull'Ipotalamo



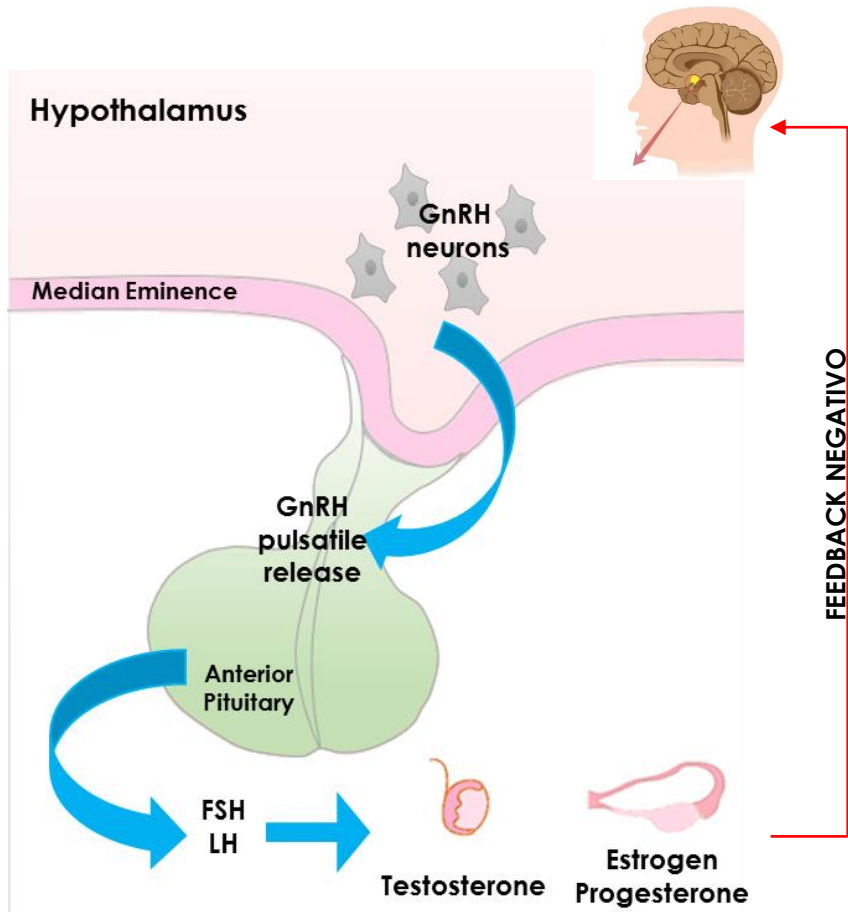
Graham G. G. & Scott K.F. Mechanism of action of Paracetamol. American Journal of Therapeutics 2005
Ogoina D. Fever, fever patterns and diseases called (fever)- a review. Journal of Infection and Public Health 2011
Blecharz-Klin K. et al Hypothalamus-Response to early paracetamol exposure in male rats offspring. International Journal of Developmental Neuroscience 2019

Il Paracetamolo agisce sull'Ipotalamo

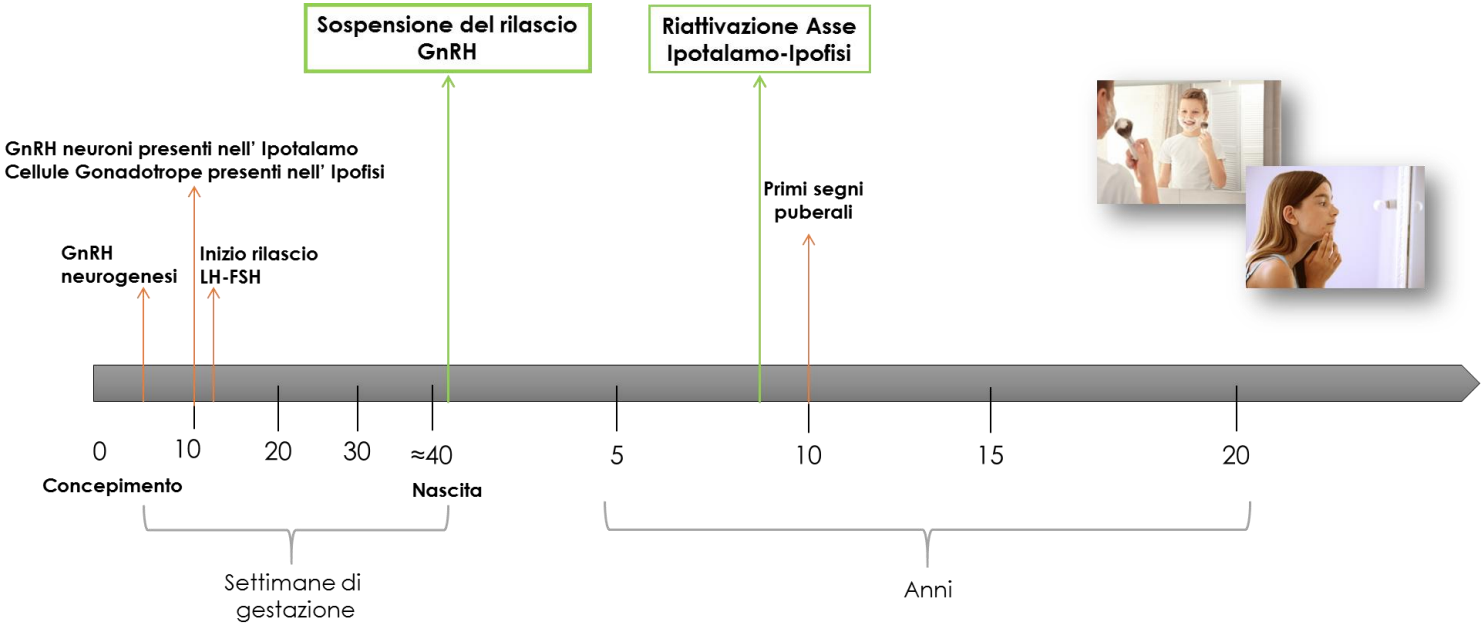
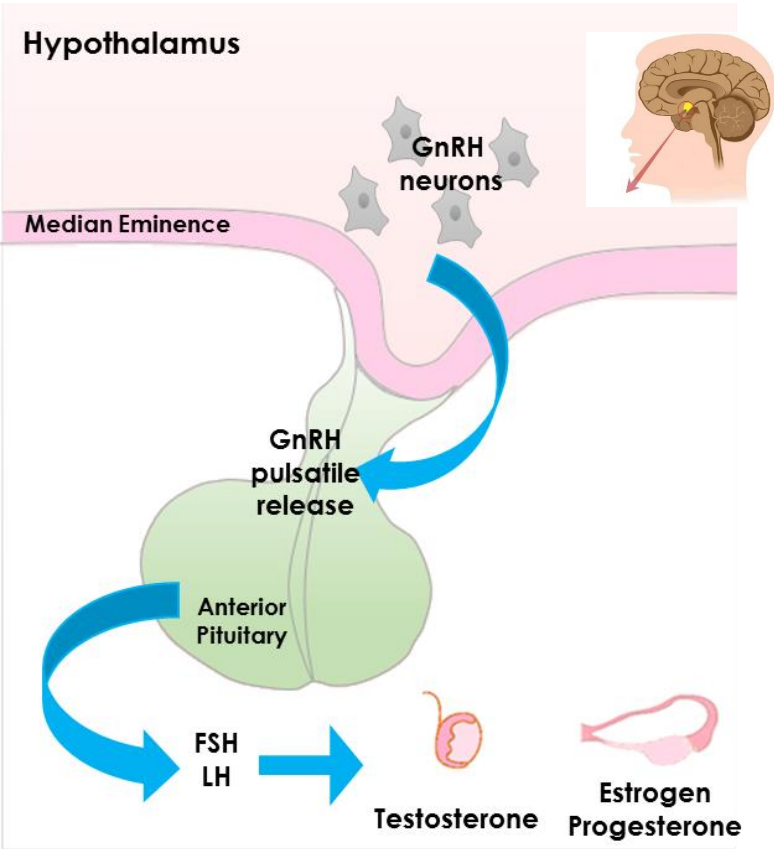


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Blecharz-Klin K. et al Hypothalamus-Response to early paracetamol exposure in male rats offspring. International Journal of Developmental Neuroscience 2019

Hypothalamo: produzione dell'Ormone di Rilascio delle Gonadotropine (GnRH)

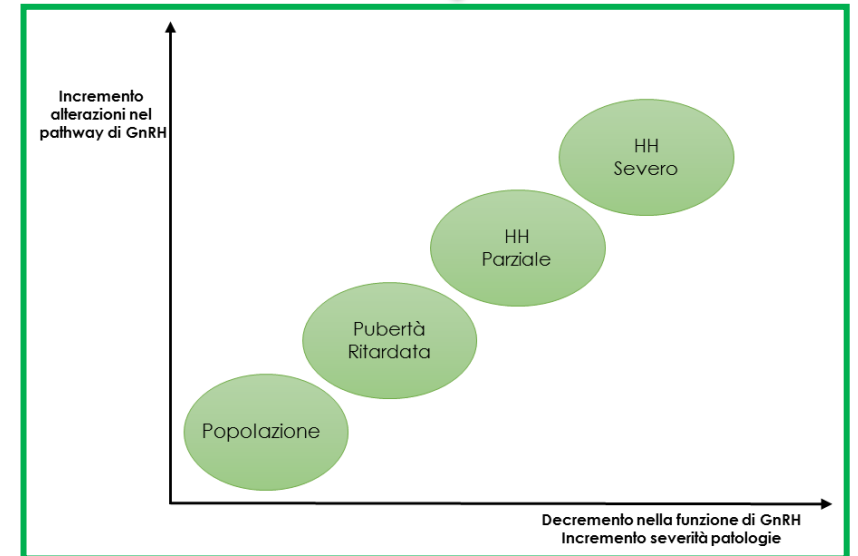
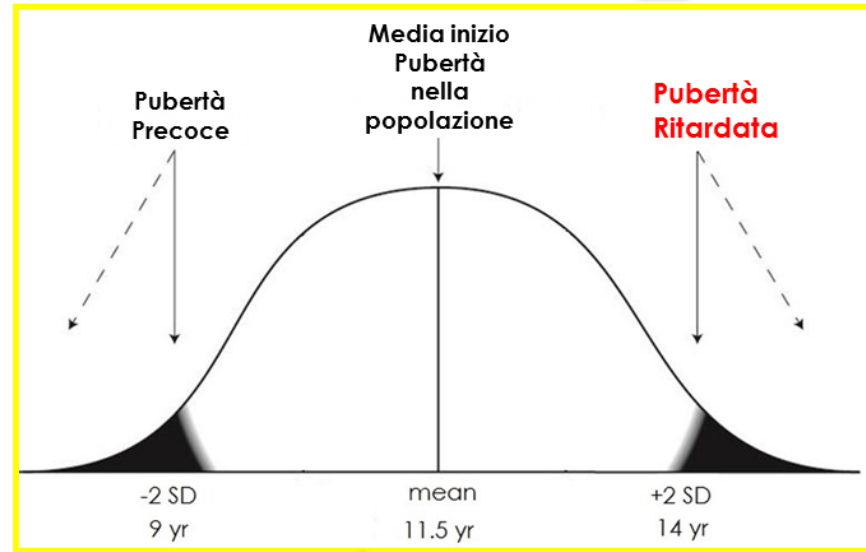
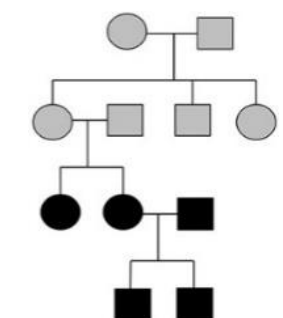


GnRH: Ormone chiave della Pubertà

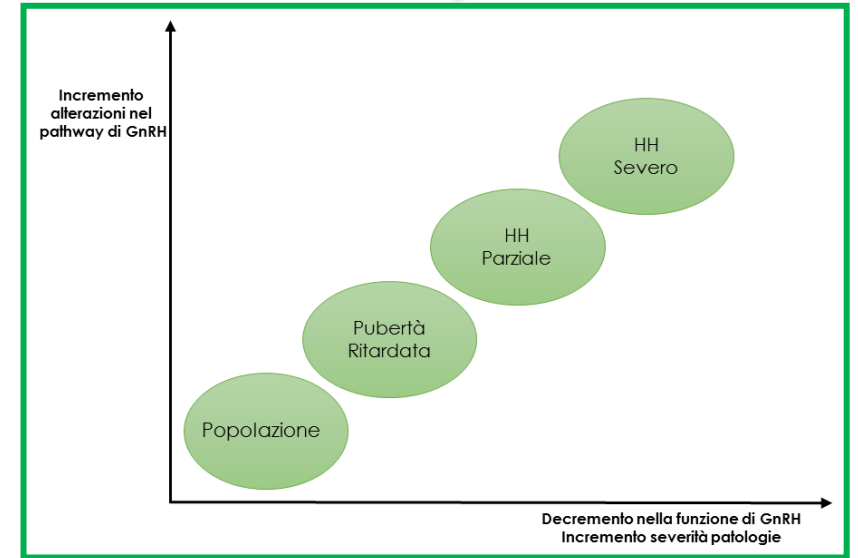
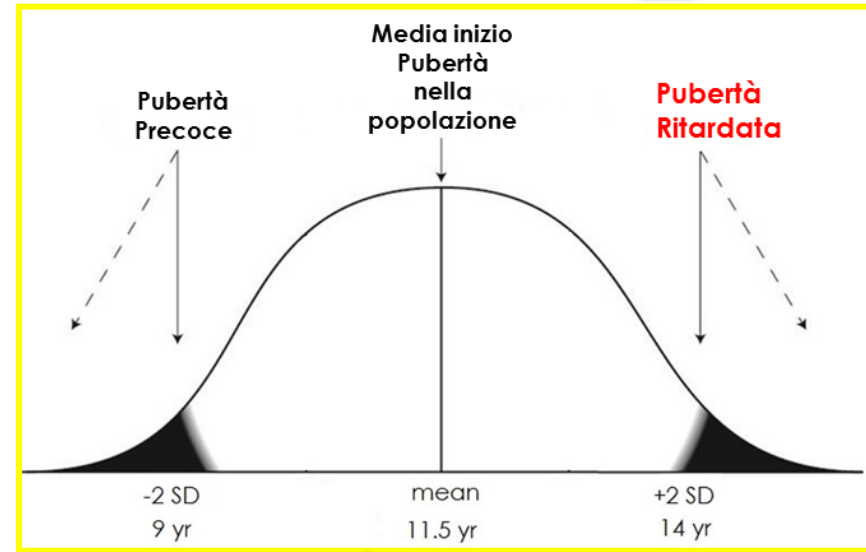
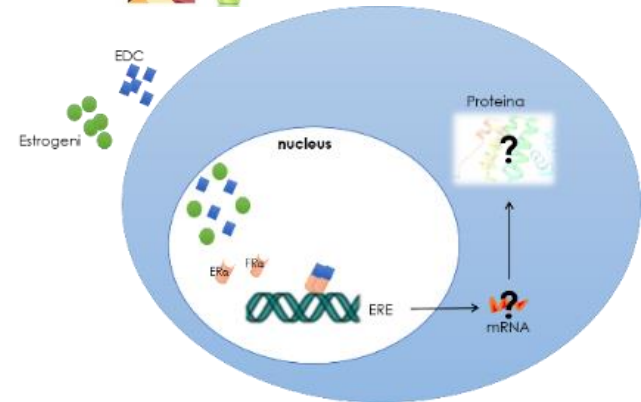


Howard S. & Dunkel L. The Genetic Basis of Delayed Puberty. Neuroendocrinology 2018
Mancini A. et al. EAP1 regulation of GnRH promoter activity is important for human pubertal timing. Human Molecular Genetics. 2019

Pubertà Ritardata



Pubertà Ritardata

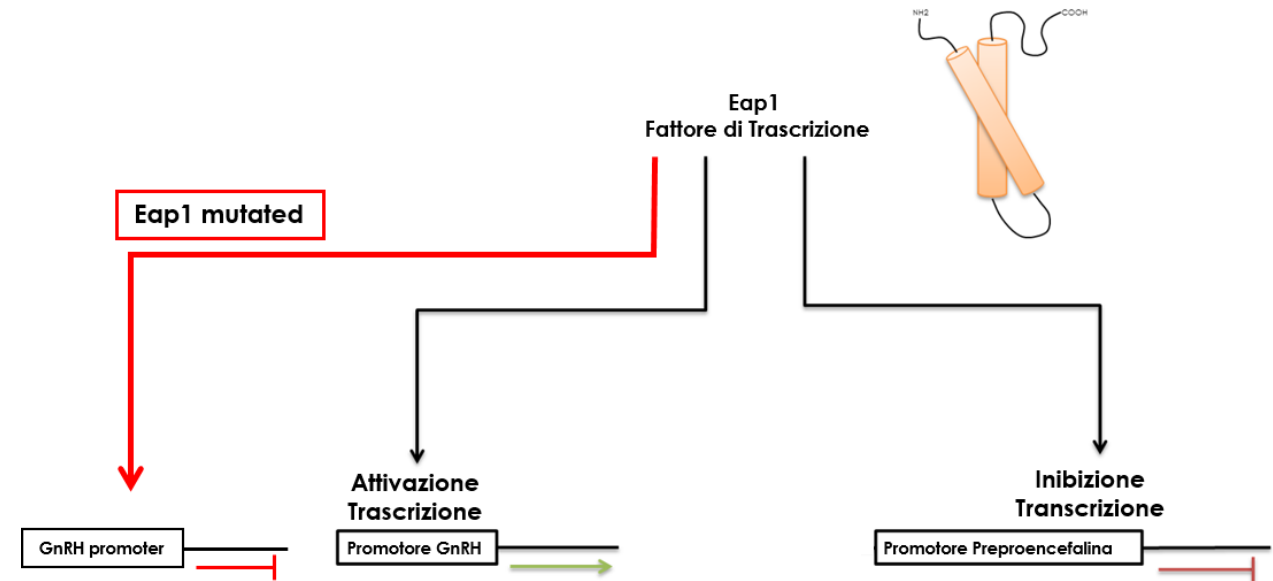


Il Paracetamolo è in grado di alterare pathways ormonali?

Reduced testis DNA synthesis resulting in decrease testicular weight, decreased number of spermatocytes and deterioration of sperm chromatin structures	Rats	Wiger R. <i>et al</i> 1995
Decreased risk of ovarian cancer	Humans	Cramer D.W. <i>et al</i> 1998a
Reduced gonadotropin and estradiol (E2) levels	Humans	Cramer D.W. <i>et al</i> 1998b
Decreased E2- dependent vitellogenin mRNA and protein production in a concentration-dependent manner	Cultured trout liver cells	Miller M.R <i>et al</i> 1999
Reduced E2-induced uterine peroxidase activity and nuclear progesterone receptor protein levels	Immature mice	R Patel & R J Rosengren 2001
Stimulated proliferation of ER-positive breast cancer cells	Cell culture	Harnagea-Theophilus E. <i>et al</i> 1998 Harnagea-Theophilus E. <i>et al</i> 1999a Harnagea-Theophilus E. <i>et al</i> 1999b
Acetaminophen exhibits weak antiestrogenic activity	Human Endometrial Adenocarcinoma (Ischikawa)cells	Dowdy J. <i>et al</i> 2003
Lower levels of testosterone produced by the foetal Leydig cells	Ex vivo organotypic culture system (rat)	Kristensen D. M. <i>et al</i> 2012
Children exposed to long-term use of paracetamol during pregnancy had substantially adverse developmental outcomes at 3 years of age	Sibling-Controlled Cohort Study	Brandlistuen R.E. <i>et al</i> 2013
Analgesics at concentrations relevant to human exposure cause endocrine disturbances in the fetal testis	<i>in vitro</i> system based on the culture of human foetal testes	Mazaud-Guittot S. <i>et al</i> 2013
Protracted use of acetaminophen (1 week) may suppress foetal testosterone production	Human fetal testis in a xenograft model (mice)	van den Driesche S. ; Macdonald J. 2015
Reduced F1 foetal germ cell number both in F/M and altered foetal germ cell development. F2 exhibited also sex-specific changes	Rats	Dean A. <i>et al</i> 2016
Shortening of the anogenital distance in adult offspring; ovaries with diminished follicle reserve and reduced fertility; reduced gonocyte number	Pregnant dams	Bak Holm J. <i>et al</i> 2016
Tendency towards slightly earlier attainment of almost all studied markers of female pubertal development with increasing number of weeks of exposure	Nationwide Puberty Cohort	Ernst A. <i>et al</i> 2019

Mutazioni nel gene *Eap1* bloccano la trascrizione di GnRH

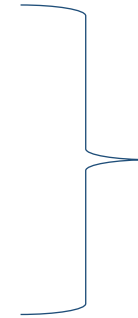
- *Eap1* mRNA aumenta durante la Pubertà
- Mutazioni nel gene di *Eap1* causano la Pubertà Ritardata



Il Paracetamolo modifica la funzione di questo fattore di trascrizione?

Ipotesi

- 1) Paracetamolo altera la trascrizione di *GnRH*?
- 2) Paracetamolo altera l'attività del fattore di trascrizione Eap1 ?
- 3) Il Paracetamolo è in grado di legarsi ad ER α ?

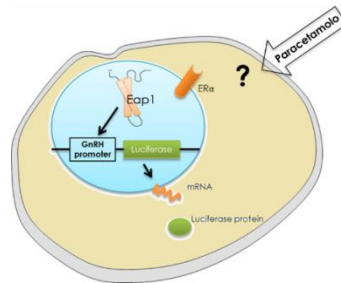


**Il Paracetamolo
si comporta
come ED?**

Luciferasi

HEK 293T

Analisi attivazione del promotore di GnRH tramite Eap1

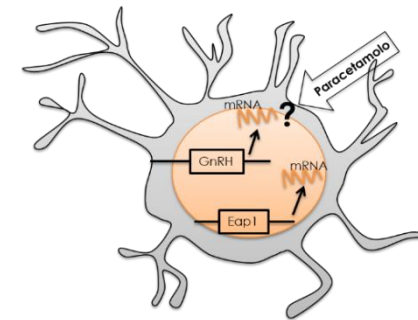


- Cellule embrionali renali
- ER α Trasfettato o no

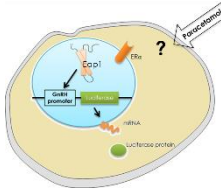
Real-Time PCR

GT1-7 cells

Analisi dell'espressione di *GnRH* *Eap1*



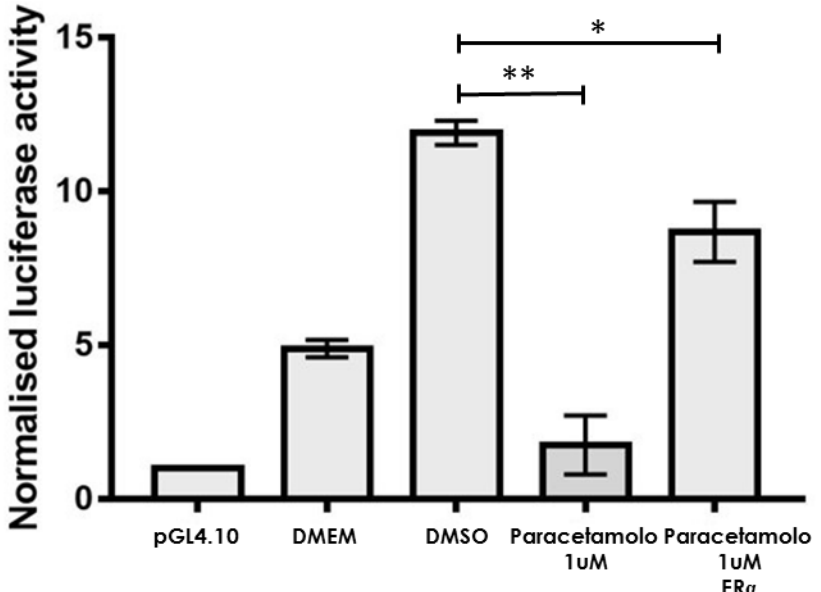
- Modello di Neuroni maturi
- GnRH, Eap1, ER α endogeni



RISULTATI LUCIFERASI:

Esposizione cellule Hek 293T ad 1uM Paracetamolo (in DMSO)

- Paracetamolo inibisce il segnale della luciferasi
- Paracetamolo inibisce il segnale maggiormente in mancanza di ER α
- DMSO mostra un effetto quando presente da solo



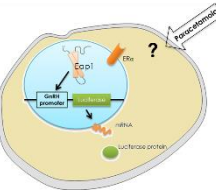
pGL4.10[luc]GnRH	+	+	+	+	+
Eap1 WT	-	+	+	+	+
ER α	-	+	+	-	+
SV-40 Renilla	+	+	+	+	+
pBlue	+	-	-	+	-

TOXICOLOGICAL SCIENCES 72, 57–65 (2003)
 DOI: 10.1093/toxsci/kg005
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Acetaminophen Exhibits Weak Antiestrogenic Activity in Human Endometrial Adenocarcinoma (Ishikawa) Cells

Janet Dowdy,* Stacey Brower,* and Michael R. Miller*†‡¹

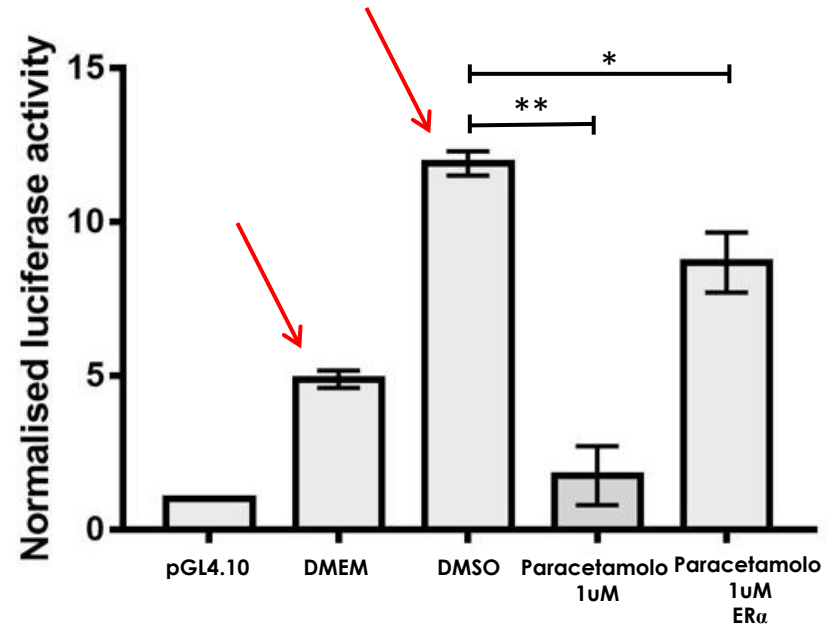
*Department of Biochemistry and Molecular Pharmacology and †Mary Babb Randolph Cancer Center, West Virginia University Health Sciences Center, P.O. Box 9142, Morgantown, West Virginia 26506–9142; and ‡National Institute of Occupational Safety and Health, Health Effects Laboratory Division, Morgantown, West Virginia 26505



RISULTATI LUCIFERASI:

Esposizione cellule Hek 293T ad 1uM Paracetamolo (in DMSO)

- DMSO mostra un effetto quando presente da solo nel terreno di cultura

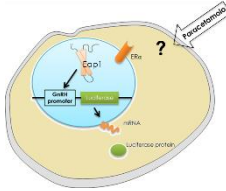
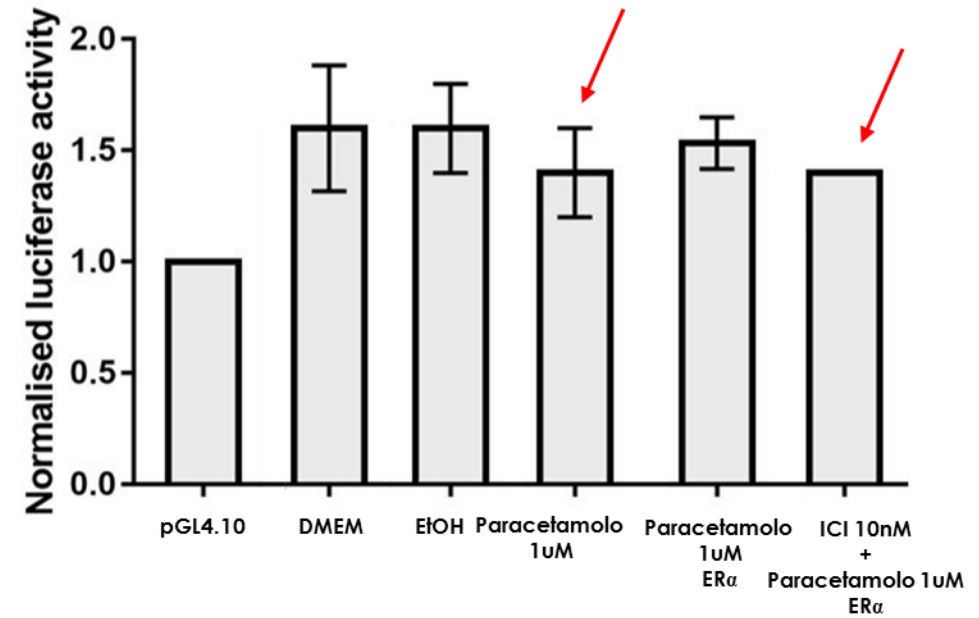


pGL4.10[luc]GnRH	+	+	+	+	+
Eap1 WT	-	+	+	+	+
ERα	-	+	+	-	+
SV-40 Renilla	+	+	+	+	+
pBlue	+	-	-	+	-

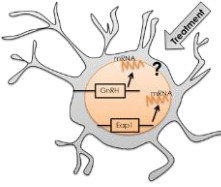
RISULTATI LUCIFERASI:

Esposizione cellule Hek 293T ad 1 μ M Paracetamolo (in EtOH)

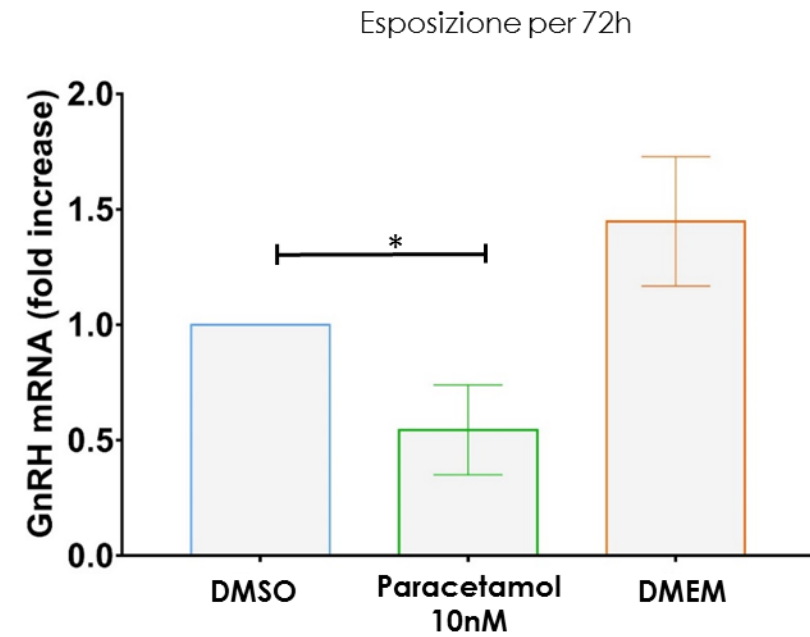
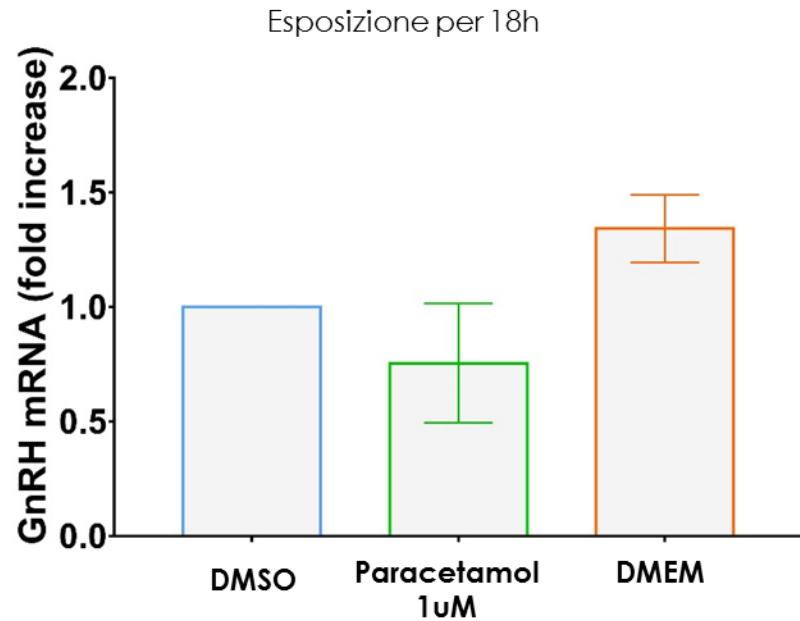
- Il segnale della luciferasi risulta diminuito in assenza di ER α
- ICI down-regolatore di ER α



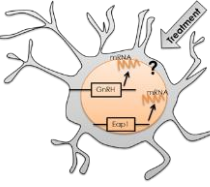
pGL4.10[luc]GnRH	+	+	+	+	+	+
Eap1 WT	-	+	+	+	+	+
ER α	-	+	+	-	+	+
SV-40 Renilla	+	+	+	+	+	+
pBlue	+	-	-	+	-	-



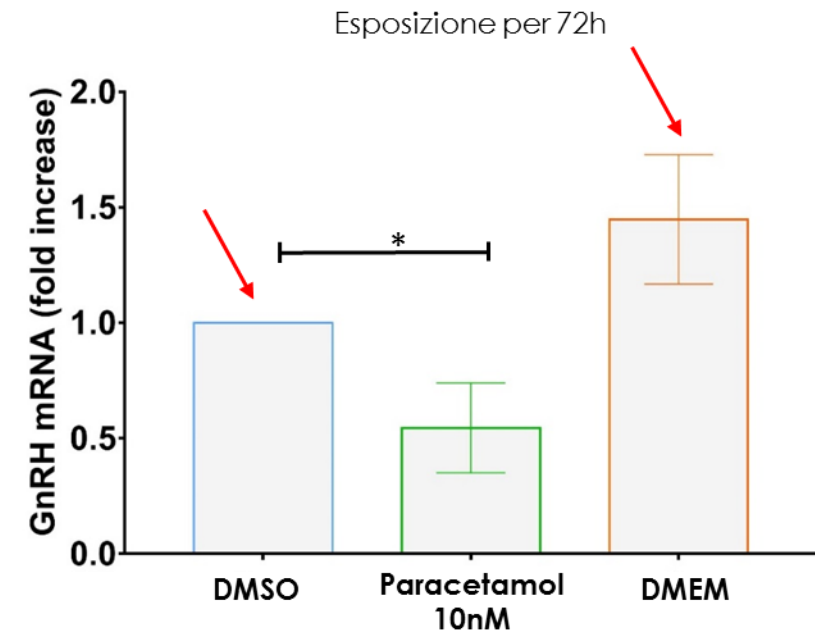
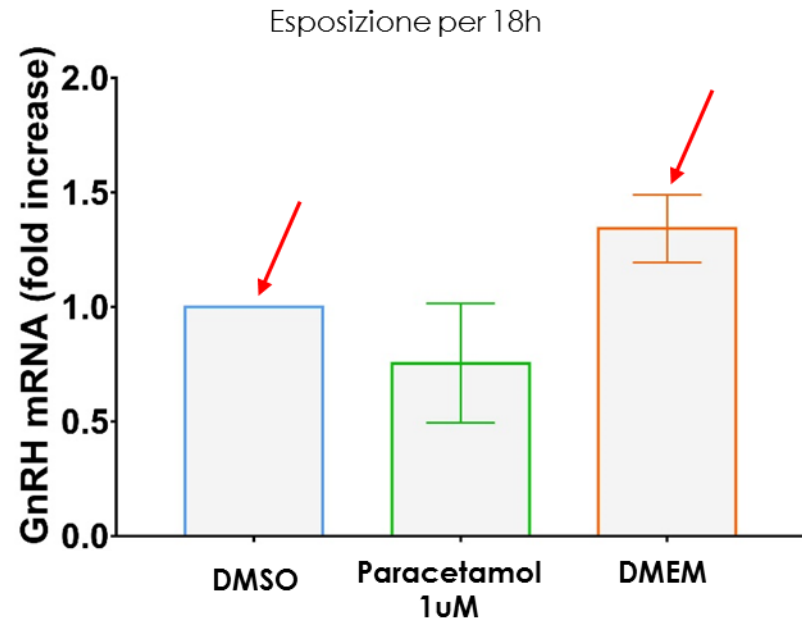
GnRH mRNA: cellule GT 1-7 trattate con Paracetamolo (in DMSO)



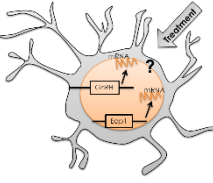
Paracetamolo inibisce l'espressione di GnRH mRNA a 72h



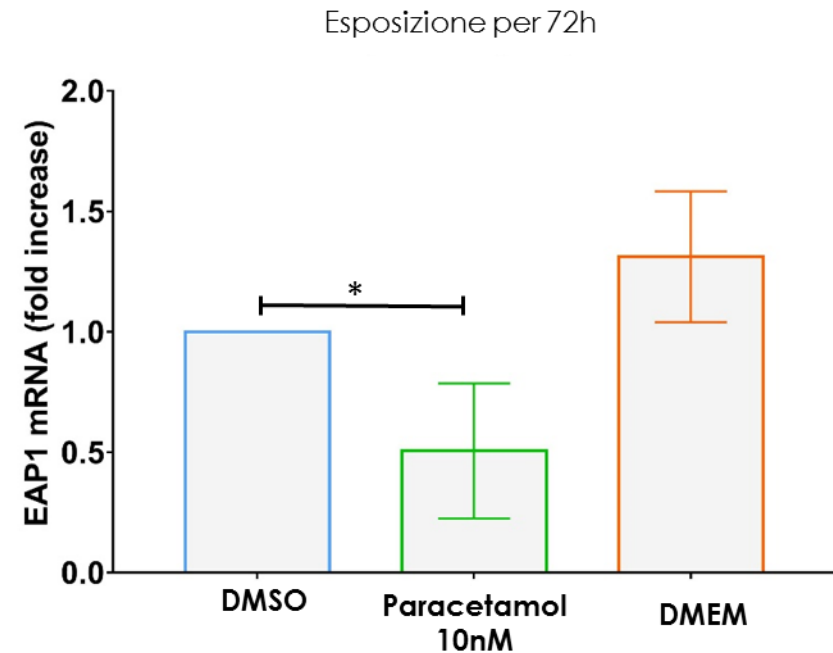
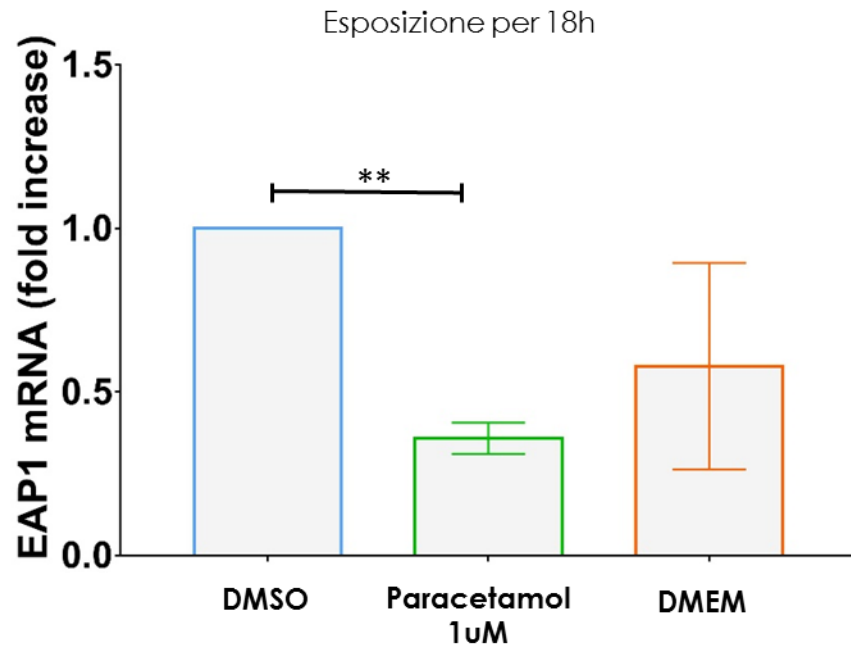
GnRH mRNA: cellule GT 1-7 trattate con Paracetamolo (in DMSO)



DMSO da solo produce un effetto



Eap1 mRNA: cellule GT 1-7 trattate con Paracetamolo (in DMSO)



Paracetamolo inibisce l'espressione di *Eap1* mRNA a 18/72h

Conclusioni

- Paracetamolo inibisce mRNA di GnRH e di Eap1, suggerendo un possibile comportamento di ED (*in vitro*)
- Paracetamolo sembra non agire tramite ER α
- DMSO sembra influenzare gli esperimenti

Prospettive future

- Ripetizione dell'esposizione con Paracetamolo in EtOH e misurazione di mRNA
- Esposizione Paracetamolo su neuroni non maturi(Gn11) e misurazione della capacità migratoria
- Analisi *in silico* dell'interazione Paracetamolo-ER α

Grazie a tutti!

WHRI Endocrinology lab

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