HYPOTHALAMIC GRK2, *VIA* GPR54, MODULATES PUBERTY ONSET

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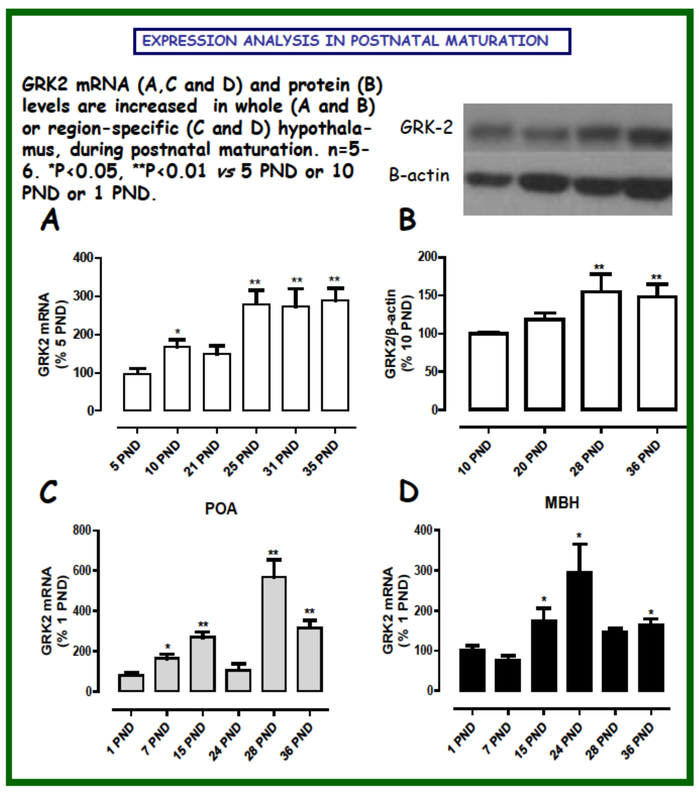
INTRODUCTION: The G protein-coupled receptor kinase 2, GRK2, is a ubiquitous serine/threonine protein kinase that is able to phosphorylate and desensitize the active form of several G protein coupled receptors (GPCR). Despite the proven role of GPCRs in the central control of reproduction, the involvement of GRK2 in these pathways remains largely unexplored. Fragmentary evidence from *in vitro* studies have suggested a potential role of GRK2 in mediating desensitization of Gpr54, the canonical receptor for kisspeptins that is abundantly expressed in GnRH neurons. Yet, although kisspeptins have been universally recognized as essential regulators of puberty and fertility, the physiological role of GRK2 in modulating kisspeptin signaling *in vivo* remains completely unexplored.

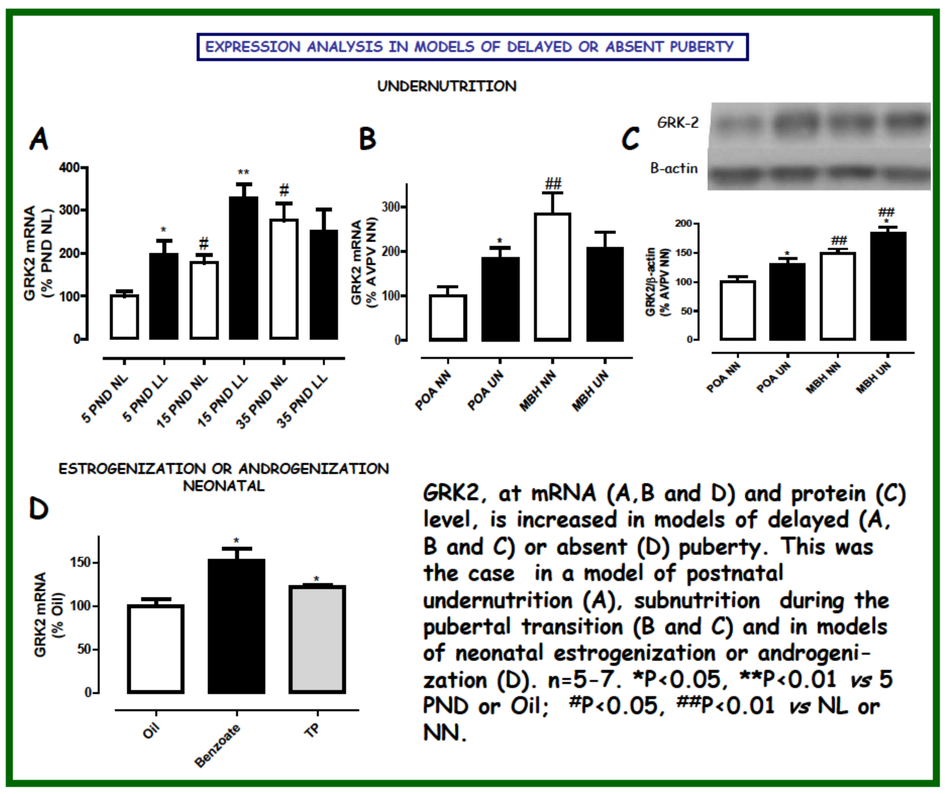
AIM: To explore the role of GRK2 in the regulation of GPR54 and neurokinin 3 receptor, at a central level, in the control of puberty

MATERIALS AND METHODS

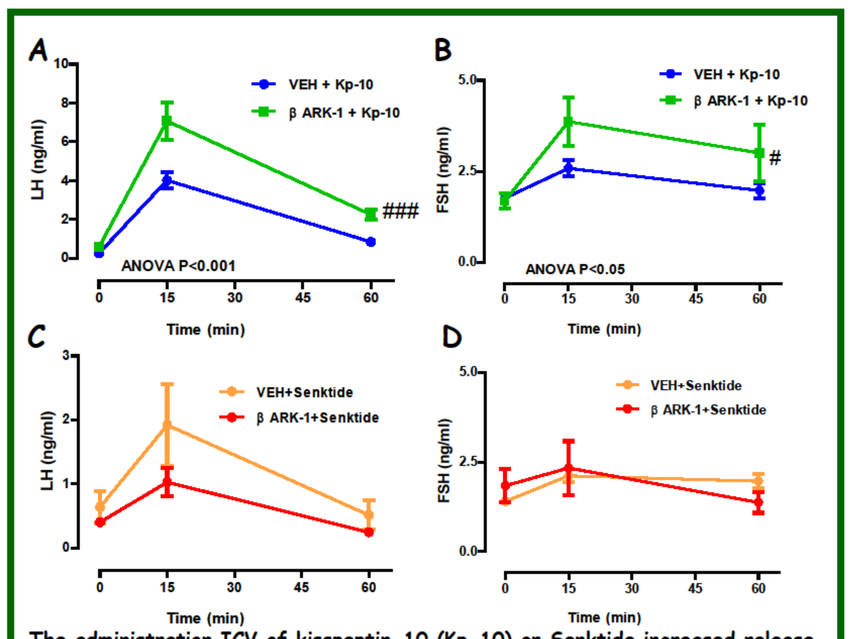
The following rat groups were used: 1) vehicle in normal litter; 2) β-ARK1, a canonical GRK2 inhibitor (6,53 nmol); in normal litter; 3) vehicle in large litter (as model of subnutrition); 4) β ARK-1 (6,53 nmol) in large litter. The animals were treated from postnatal day 25 until sacrifice. Food intake, body weight, pubertal parameters (vaginal opening and first estrus), uterus and ovary weights and blood samples was evaluated. GRK2 mRNA and protein levels in hypothalamus were determined by qRT-PCR (mRNA expression) and Western-blot (protein expression). Acute tests were performed by injection ICV of GRK2 inhibitor or vehicle, 13 h and 1 h, before the injection of Kisspeptin-10 (agonist to GPR54) or Senktide (agonist to NK 3 receptor) and blood samples were taken at 0 min (basal), 15 min and 60 min thereafter.

RESULTS

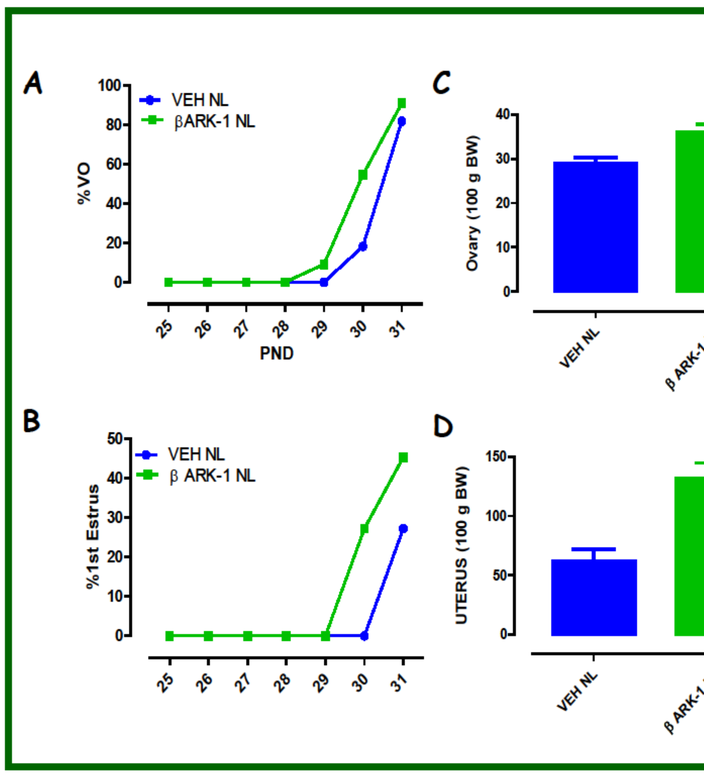




GRK2, at mRNA (A,B and D) and protein (C) level, is increased in models of delayed (A, B and C) or absent (D) puberty. This was the case in a model of postnatal undernutrition (A), subnutrition during the pubertal transition (B and C) and in models of neonatal estrogenization or androgenization (D). n=5-7. *P<0.05, **P<0.01 *vs* 5 PND or Oil; #P<0.05, ##P<0.01 *vs* NL or NN.



The administration ICV of kisspeptin-10 (Kp-10) or Senktide increased release of LH and FSH (A,B, C and D). However, when animals were pretreated with β ARK-1 (GRK2 inhibitor), LH and FSH responses to kp-10 were augmented (A and B). In contrast, the administration of Senktide and β ARK-1 did not modify LH an FSH secretion (C and D). n=5-7. #P<0.05, ###P<0.001 vs VEH + Kp-10.



In control female rats, treatment with β ARK-1 induced an advance of puberty onset, as shown by earlier vaginal opening (A) and first estrus (B). Moreover, this was associated with an increase in ovary (C) and uterus (D) weights and an increase in LH (E) and FSH (F) levels. n=5-9. *P<0.05, **P<0.01, ***P<0.001 *vs* VEH NL.

In females subjected to postnatal subnutrition, reared in large litters (LL), the expected delay in pubertal parameters was detected, as shown by delayed vaginal opening (G) and first estrus (H). This was accompanied by a decrease in the weight of ovaries (I) and uterus (J). However, the treatment with β ARK-1 partially restored the delay in puberty (G and H) and normalized uterus (J) and ovary (I) weights, without changes in the levels of LH (K) and FSH (L). n=5-9. *P<0.05 *vs* VEH LL. #P<0.05 *vs* VEH NL.

CONCLUSIONS

Our results demonstrate that GRK2 regulates hypothalamic Gpr54 signaling *in vivo* and provide conclusive evidence for a crucial role of GRK2 in the fine tuning of pubertal timing, likely *via* modulation of kisspeptin actions, in normal and metabolically or hormonally compromised conditions.

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