Kisspeptin increases during reproductive aging and is regulated by sympathetic nerve system.

Fernandois D, Márquez R, Lara HE, Paredes AH.

Laboratory of Neurobiochemistry. Dept. of Biochemistry and Molecular Biology, Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Chile

Introduction

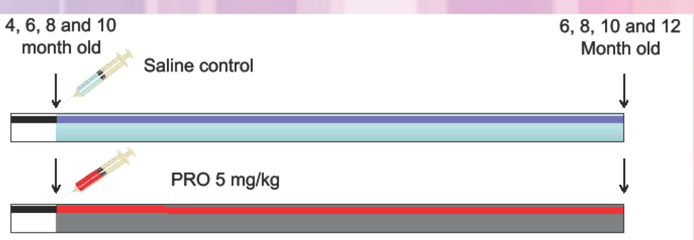
According with the World Bank reproductive health indicators, in the last 13 years the world rate of fertility has declined, especially in the upper middle and high income countries (rate of 1,9 and 1,7 birth per women respectively). One possible cause of this is that in the present decade couples have decided to delay motherhood when women are close to the subfertility window (near 35 years). This also has increased consultation in fertilization programs. Furthermore, recent evidence indicates that an early menarche in women is associated with premature depletion of ovarian follicular reserve (Weghofer A, 2013). Because of this, understanding the mechanism of follicular development during the subfertility period is now critical. In this regard, it has been described that during the subfertility period occurs an increased loss of follicular reserve (Faddy et al, 1992) and an increase in the ovarian innervation (Heider et al, 2001). Particularly to this, our group is interested in identifying key actors of follicular development, and its alteration during the subfertility period concerning to the change in nervous system functioning. To asses this we use Sprague-Dawley rats, which during their reproductive senescence (8 to 12 months) has an increase in ovarian sympathetic tone. This increased sympathetic tone, has been associated with a decrease in corpora lutea, antral follicles and an increase in follicular cysts (Acuña et al, 2009). Also we described that young ovaries have the ability to increase kisspeptin production under a β-adrenergic stimulation using isoproterenol (Ricu et al, 2012). Since kisspeptin is a peptide that is present in the ovary, and yet has not been associated to a particular ovarian function, we want to investigate if kisspeptin changes during ageing. Our results show that kisspeptin is increased during ageing in the ovary, and when we blocked the sympathetic tone the kisspeptin levels decreased. This can open the possibility to consider kisspeptin as a new pharmacological target to consider in females within the subfertility period.

Purpose

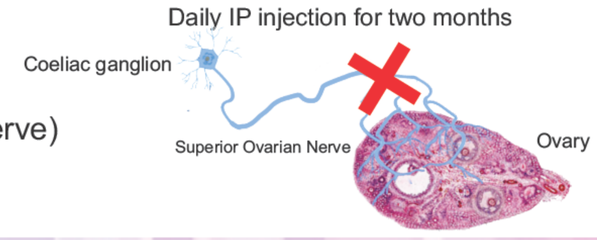
The aim of this study is to asses if kisspeptin levels are regulated by the adrenergic nervous system in the ovary using *in vivo* experiments.

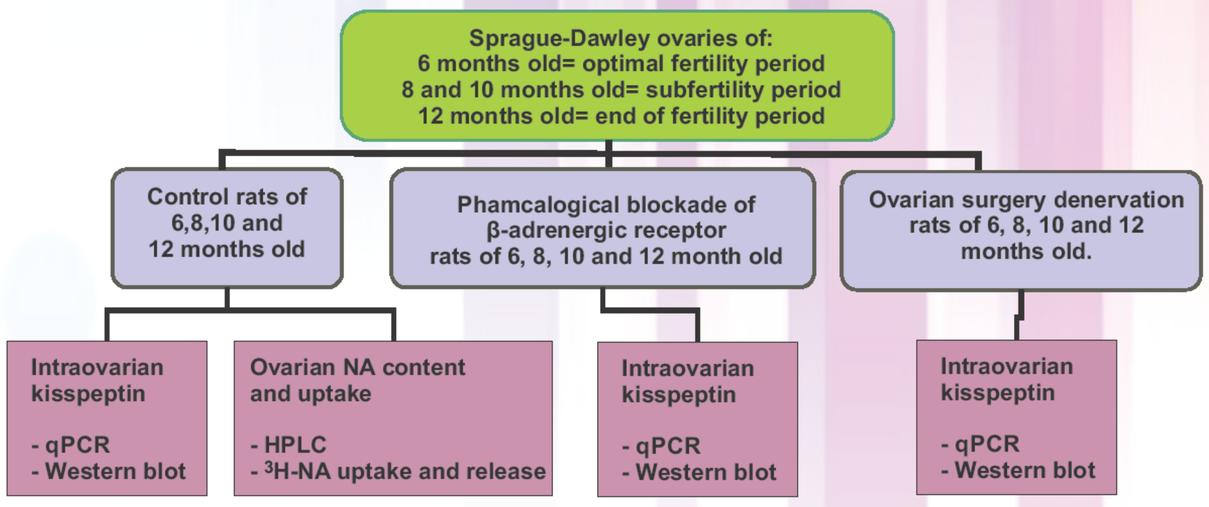
Methods

1.- Pharmacological Blockade (Daily Intra peritoneal injection of Propranolol a β-adrenergic antagonist)



2.- Surgical Denervation (Resection of superior ovarian nerve)





Results

1 Ovarian kisspeptin is increased during ageing.

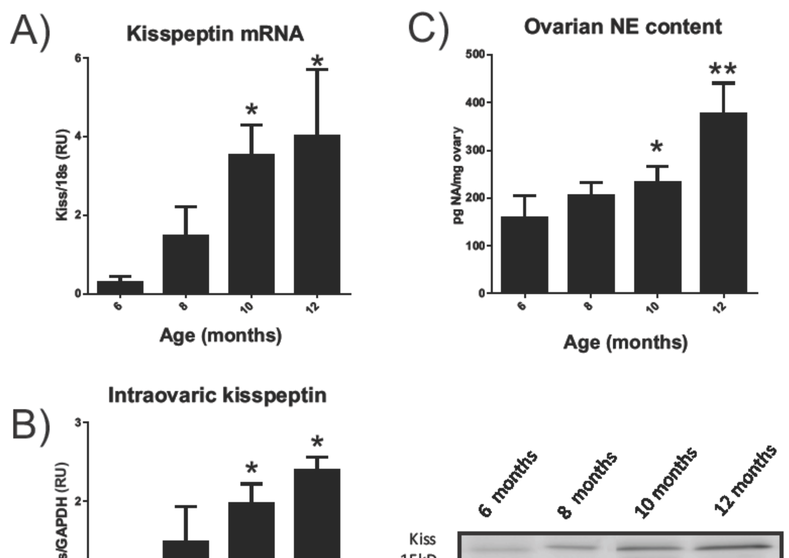


Figure 1.- Kisspeptin increases during reproductive aging. A) Kisspeptin transcript levels in the ovary of Sprague-Dawley rats quantified by RT-qPCR. mRNA levels were normalized with 18S gene. N=6 (minimum) for each group B) Protein levels quantified by western blotting, each sample was assessed 3 times in triplicated. Kisspeptin values were normalized with GAPDH protein. Pixels were counted with ImageJ N=4 . C) Ovarian noradrenaline content measured by HPLC. N=4. The results are plotted as the mean ± SEM. Statistics: for graph A the significance was obtained with a Kruskal-Wallis test followed by Dunn's multiple comparison. For B and C significance was obtained with a One-way ANOVA, followed by the multiple comparison test of Fisher's LSD. p <0.05 = * and p<0,01 = **.

2 Kisspeptin is correlated with adrenergic activity in the ovary.

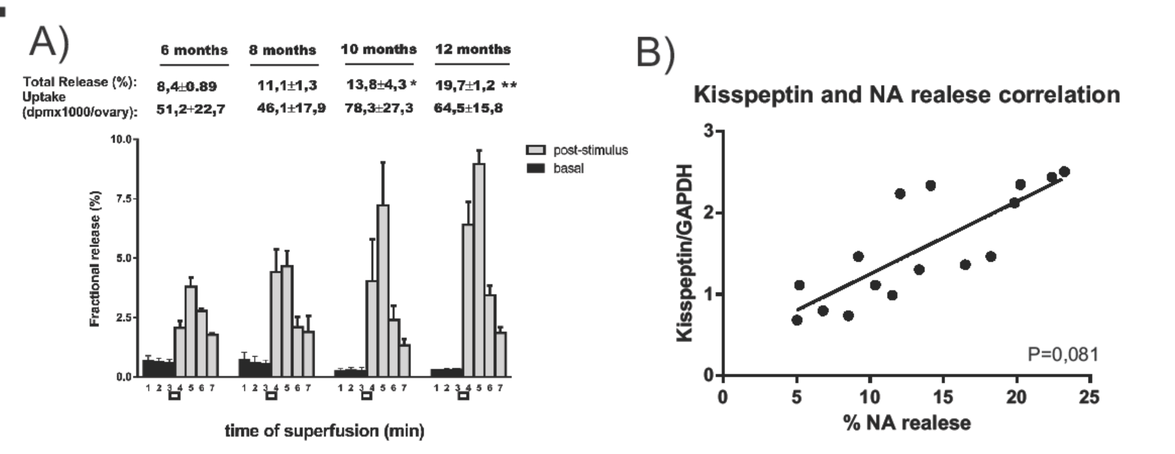


Figure 2.- Correlation of kisspeptin with noradrenaline. A) Release of noradrenaline was induced by electric-induced depolarisation. Results are expressed as a total 3H-NA released by electric depolarisation and uptake of NA. B) Correlation between kisspeptin protein level showed in the Fig 1B and NA release showed in A. The graphs era plotted as the mean ± SEM. Correlation was obtained with a Pearson test. p <0.05 = * and p<0,01 = **.

3 Ovarian kisspeptin diminish under a β-adrenergic blockade with propranolol.

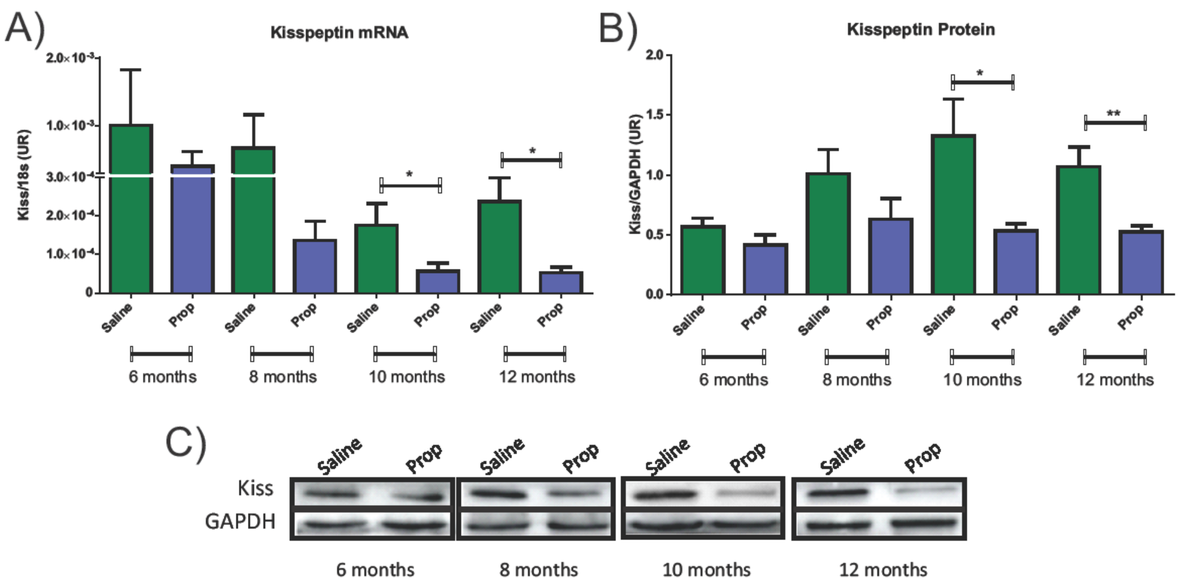


Figure 3.- Kisspeptin diminish under a b-adrenergic blockade. A) Kisspeptin transcript levels in ovaries under a b-adrenergic blockade with propranolol (5mg/Kg) or saline control vehicle for 2 months. mRNA levels were quantified by a RT-qPCR and normalized with 18S gene. N=6 (minimum) for each group B) Protein levels quantified by western blotting, each sample was assessed 3 times in triplicated. Kisspeptin values were normalized with GAPDH protein. Pixels were counted with ImageJ, N=5 (minimum) . C) Representative image of western blotting of each age condition. The results are plotted as the mean ± SEM. Significance was obtained with a Student t-test, between saline control and Propranolol for each age. p <0.05 = * and p<0,01 = **.

4 Ovarian kisspeptin diminish under a surgical denervation of ovarian superior nerve (SONX).

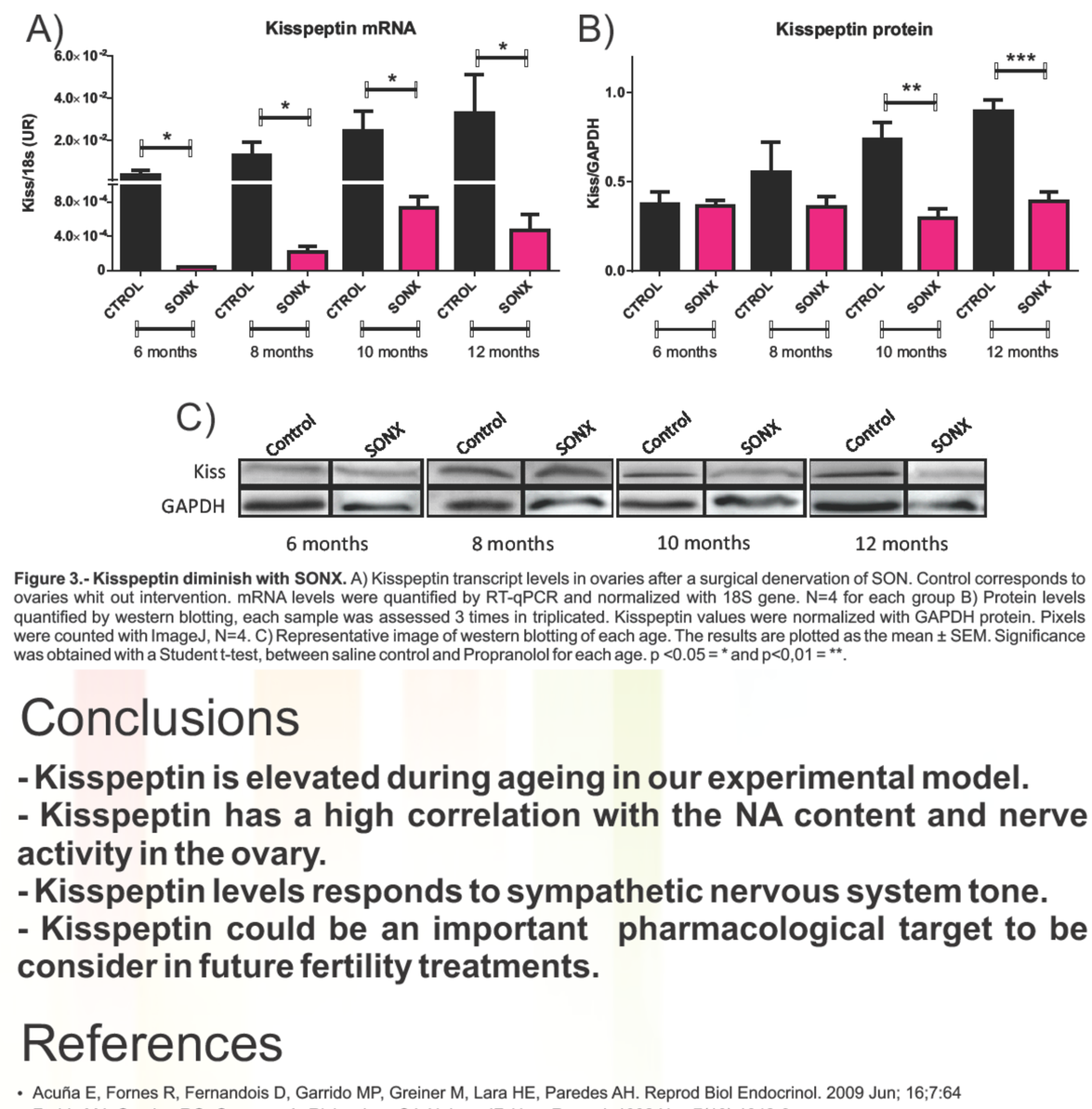


Figure 3.- Kisspeptin diminish with SONX. A) Kisspeptin transcript levels in ovaries after a surgical denervation of SON. Control corresponds to ovaries whit out intervention. mRNA levels were quantified by RT-qPCR and normalized with 18S gene. N=4 for each group B) Protein levels quantified by western blotting, each sample was assessed 3 times in triplicated. Kisspeptin values were normalized with GAPDH protein. Pixels were counted with ImageJ, N=4. C) Representative image of western blotting of each age. The results are plotted as the mean ± SEM. Significance was obtained with a Student t-test, between saline control and Propranolol for each age. p <0.05 = * and p<0,01 = **.

Conclusions

- **Kisspeptin is elevated during ageing in our experimental model.**
- **Kisspeptin has a high correlation with the NA content and nerve activity in the ovary.**
- **Kisspeptin levels responds to sympathetic nervous system tone.**
- **Kisspeptin could be an important pharmacological target to be consider in future fertility treatments.**

References

- Acuña E, Fornes R, Fernandois D, Garrido MP, Greiner M, Lara HE, Paredes AH. Reprod Biol Endocrinol. 2009 Jun; 16;7:64
- Faddy MJ, Gosden RG, Gougeon A, Richardson SJ, Nelson JF. Hum Reprod. 1992 Nov;7(10):1342-6.
- Heider U, Pedal I, Spanel-Borowski K. Fertil Steril. 2001 Jun;75(6):1141-7.
- Ricu MA, Ramirez VD, Paredes AH, Lara HE. Endocrinology. 2012 Oct;153(10):4966-77
- Weghofer A, Kim A, Barad DH, Gleicher N. Fertil Steril. 2013 Oct;100(4):1039-43

17th European Congress of Endocrinology 16-20 May 2015, Dublin Ireland Grants: FONDECYT 1120147 (AP), Beca Doctorado CONICYT 21120454 (DF)