Research Journal of Pharmacology 1 (1): 14-18, 2007

The Pharmacological Effect of Small Doses of an Engogenous Opioid Antagonist on the Pulsatile Secretion of LH in the Goat During Anoestrus

V.O.J. Fuentes, P. Fuentes-Castro, M. Fuentes-Castro, A. Bernal-Canseco,
R. Orozco-Hernández and J.J. Uribe-Gómez
Centro Universitario de los Altos, Universidad de Guadalajara,
Hospital PEMEX de Alta Especialidad, México, D.F. México

Abstract: With the objective of studying the pattern of LH secretion in the anoestrus goat and the pharmacological effect of a subcutaneous implant containing 15 mg naloxone on the plasmatic concentrations of LH in the goat during the anoestrus period. 15 Alpine does were selected, body weight and age averaged 35.8 kg and 8 months respectively all goats chosen were considered sexually mature. The experiment was carried out during the months of May and June 2005 during seasonal anestrus in this latitude (19° 13´North). In groups of five chosen at random, they were allocated as follows: Group A was used as control injected i.m. with 2 mL saline at 12 h intervals for 15 days, group B received a subcutaneous implant of 15 mg naloxone. And group C received an i.m. injection of 0.5 mg naloxone i.m. at 12 h intervals (8:00 and 20:00 h) for 15 days. In all groups LH values were similar to those observed in day 0, 5 and 10 no difference was detected between groups but treated groups showed signicant surges of LH. On the 15th day of treatment a significant difference was observed between treated animals (implant and injection), significant surges of LH were observed in treated groups; On day 20 implanted animals showed higher levels of LH than injected and control groups with a significant surge detected during the sampling period. In control goats basal LH levels were maintained during the period of study. It was concluded that the administration of naloxone by implant induced LH increases significantly greater as compared with the injection group and that the concentration of LH in control groups it was similar to the one reported in sheep.

Key words: Ewe, LH, naloxone, implant, goat, antagonist

INTRODUCTION

The survival of species is determined through their capacity for reproduction and this event is controlled by the interaction of different biological systems with in each species. Reproductive behaviour is under the control of external and internal factors. All of which permit birth to happen during the appropriate time of the year, in order to insure the survival of the new born (Fabre-Nys, 1993; Mori, 1989). The ewe and goat are good examples, these small ruminants have a period of anoestrus followed by a period of sexual activity (estrus). The expression of sexual behaviour is stimulated during the beginning of the short days, while the period of anoestrus is onset during the long days (Fuentes, 1988). This behaviour is more determinant in the north hemisphere, where the European breeds originated (Chemineau, 1988). In countries near the equator photoperiod changes are not so marked, when European goats are raised near the equator sexual behaviour is not changed (Delgadillo *et al*., 2004).

The period of anestrus is of great importance for goat breeders because during this season productivity is not optimal and many research teams are studying the mechanisms that determine the presence of periods of anestrus and estrus, using small ruminants as a model for such observations (Currie, 1991; Chemineau, 1988; Malven, 1995; Goodman *et al*., 2006). The capacity for connecting and disconnecting the display of sexual behaviour in these species means, that there is an endogenous mechanism that through external and internal sensors are able to control the function of the hypothalamic- hypophisial-gonadal axis. Modulating the secretion of the gonadotrophic hormones such as LH (Edqvist, 1993; Fabre-Nys, 1993). During anoestrus the pulsatile secretion of LH is diminished (Schall *et al*., 1991) and during the breeding season pulsatile secretion of LH is increased.

The secretion of gonadotrophins is under the influence of releasing hormones that are originated in the median eminence of the hypothalamus. Furthermore,

Corresponding Author: V.O.J. Fuentes, Centro Universitario de los Altos, Universidad de Guadalajara,
Hospital PEMEX de Alta Especialidad, México, D.F. México

releasing hormones are secreted at rates determined by the influence of central nervous transmitters and neurohormones. Among the latter there is evidence that melatonin, dopamine and peptide opioids are important modulators of GnRH secretion (Currie, 1989; 1991; Malven, 1995; Fuentes, 1998).

Pharmacological manipulation of opioidergic receptors with opioid antagonists induce changes in the pattern of gonadotrophic hormones secretion in several species (Brooks, 1986; Cosgrove, 1993; Fuentes, 1988; Grossman, 1988). While the use of small doses of opioid antagonists with selective affinity for μ-endorphinergic receptors, such as naloxone, induced changes in the sexual behaviour and blood hormones levels of sheep and goats (Fuentes *et al*., 1988; 2006).

The administration of naloxone is limited by the pharmacological characteristic of its absorption and distribution through the experimental animal body. Naloxone is administered solely by the parenteral route, therefore all previous experiments with this opioid antagonist have being carried out using the intramuscular and intravenous routes. It was considered of interest to study: first: in the anoestrus goat the pattern of LH secretion and 2nd the pharmacological effect of a subcutaneous implant containing 15 mg naloxone and compare this effect with injected naloxone on the plasmatic concentrations of LH of the goat during the anoestrus period.

MATERIALS AND METHODS

From a commercial flock, 15 sexually mature young Alpine does were selected, body weight and age averaged 35.8 kg and 8 months respectively, after clinical observation all goats used were considered sexually mature. The experiment was carried out during the months of May and June when seasonal anestrus is displayed in this latitude (19° 13´North). They were housed in open paddocks and fed oat hay ad libitum supplemented with 0.4 kg concentrate per animal per day with water ad libitum. In groups of five chosen at random, separated at least 400 meters one from the other to avoid biostimulation (Rekwot *et al*., 2001), they were treated as follows: Group A was used as control, injected at 12 h intervals for 15 days with 3 mL saline, group B received a subcutaneous implant behind the ear under local anaesthesia; the implant contained 15 mg naloxone in a crystalline micro cellulose pellet (total weight 250 mg, 3 mm wide 5 mm long) calculated for complete absorption in 15±2 days. And group C received an i.m. injection of 0.5 mg naloxone at 12 h intervals for 15 days (injections were given at 8:00 and 20:00 h).

To observe the effect of treatment on LH secretion, jugular blood samples (2 mL) were obtained trough an implanted catheter, sampling was carried out at 20 min intervals for 7 continuous hours on days 0, 5, 10, 15 and 21 of the duration of the experiment. Blood samples were centrifuged at 2,500 rpm for 15 min; plasma was separated in individual plastic vials and stored frozen until LH assay was carried out.

LH concentrations were determined in duplicate plasma samples using a commercial liquid phase double antibody radioimmunoassay (Mymnsa, México) with an intra and inter assay coefficients of variation of 5 and 9%. Sensitivity was 0.1 ng mL^{-1} serum. Statistical analysis for LH concentrations was studied using a descriptive method followed by an inferential screening and a variance analysis.

RESULTS

It was observed that the plasmatic concentrations of LH changed through time in all groups, LH concentrations in naloxone treated animals were greater at all times and showed more changes as compared with the control group. These changes can be appreciated observing Fig. 1 and 2, it was observed that on days 0, 5 and 10 LH concentrations were greater in naloxone treated groups. After the morning injection of naloxone it was observed that LH levels increased significantly and at the end of the sampling period they still were higher than controls.

On the 15th day of treatment a significant difference was observed between treated animals (implant and injection), significant surges of LH were observed in naloxone treated groups; while in control goats basal LH levels remained with no change.

On day 20; LH plasmatic levels were greater in the group treated with an implant as compared with goats treated with naloxone by intermittent injections and significantly greater than LH levels of the control group. In this day the group treated with an implant of naloxone displayed a significant LH surge.

An influence of time upon the plasmatic levels of LH was also observed, showing a high degree of significance ($f = 12.58$, (4.44) $p = 0.0000005$). The analysis of the influence of the pharmacological treatment upon time on the plasmatic levels of LH showed a large difference was detected when comparing the first two samples of day 0 with the samples obtained in days 10, 15 and 20 of the experiment.

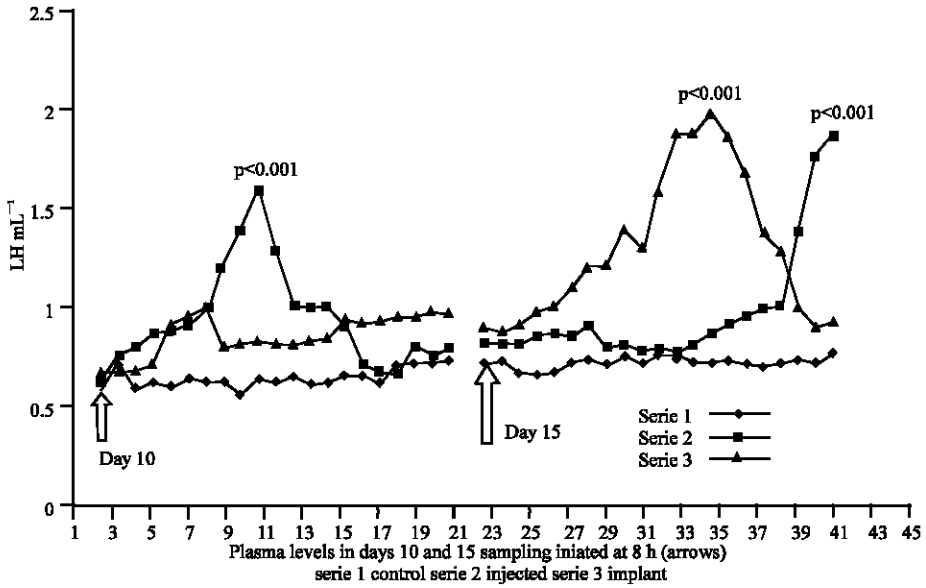

Fig. 1: The pharmacological effect of naloxone by intermitent injections (0.5 mg/12 h/15 days) and implant (15 mg)on LH pulsatile levels in anoestrous goats

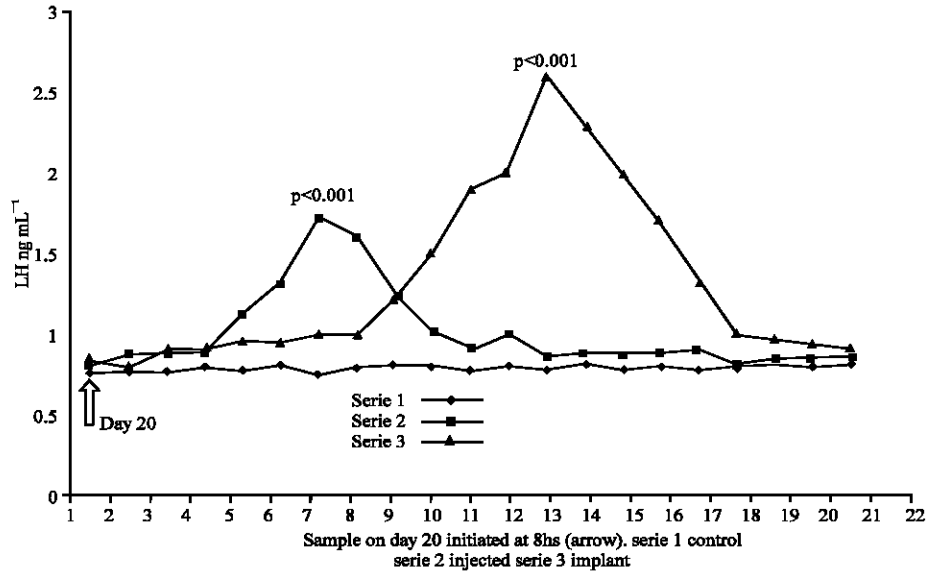

Fig 2: Pharmacological effect of naloxone administered by and injection in anoestrous goats

The statistical analysis showed that the administration of naloxone by injection or through a subcutaneous implant significantly increased plasmatic levels of LH in goats of 8 months of age during anoestrus at a 19°13' latitude north.

DISCUSSION

The observed results in this work the plasmatic concentrations of LH during the anoestrus season in goats, are in agreement with the reports of similar observations in the ewe (Currie, 1991; Fuentes, 1998; Malven, 1995)goats (Fuentes, 1997; Cheminenau, 1998).

Naloxone an opioid antagonist used as a pharmacological tool to study the interaction of endogenous opioids with sexual function, has being used by many research teams. It is necessary to mention that the dose used in those experiment was 0.5 mg kg^{-1} (Currie, 1989) in this research the dose used was chosen with the objective of administering physiological or/and

therapeutic levels (Fuentes *et al*., 2006), therefore 1.0 mg total dose per day in the goat was selected. In previous work Fuentes (1988; 1998) using the same dose (1 mg day^{-1}) has reported changes in sexual behaviour and changes in the pulsatile release of LH. In this work there was a difference in the administration route of naloxone, using an implant containing 15 mg naloxone, with the aim of providing a continuous supply of the opioid antagonist and to avoid management stress due to continuous handling as needed when naloxone was administered by intermittent daily injections.

It was observed that the pharmacological effect of naloxone administered via a subcutaneous implant produces much the same changes in LH as those observed with injections of 0.5 mg of naloxone at 12 h intervals. When Tuckey's test is used to study these changes a significant difference was observed between the plasmatic levels of control goats as compared with those of the naloxone treated animals. This observation further supports that the pharmacological effect of naloxone administered in low doses (by implant or injection) produces changes of significance in sexual behaviour and Hormone concentrations (LH).

Note should be made of the fact that the effect of the chronic or intermittent administration of low doses of naloxone does not produce an immediate effect but it takes some time for significant changes to take place and this effect was previously observed in rams and bucks (Fuentes *et al*., 1997; 1998). There is the possibility that opioid antagonists induce a paradoxical effect when administered in very low doses (Powell *et al*., 2002). Some have suggested that there is a possibility that low doses of opioid antagonists show synergistic binding with μ receptors (George *et al*., 2000). It is possible that μ receptors have different variants (Powell *et al*., 2002). Furthermore, Pasternak (2001) has reported at least seven different splice variants of the μ opioid receptor. And it is accepted that μ receptors (on which naloxone is pharmacologically very selective) are related with GnRH release. Therefore it is possible to conclude and postulate that with this results and previous work using small doses of naloxone, the opioid antagonist is interacting with μ receptors in is such a way that after a period of time they produce a facilitating effect on GnRH neurons.

This research shows three important results: One that the the pattern of LH secretion of the goat during anoestrus in this lattitude is similar to the one reported in ewes. Second, that the administration of naloxone trough a subcutaneous implant produces the same effect as the one observed when naloxone is administered by intermittent injections. And three, the need of further research using low doses of opioid antagonists to further understand their interaction with opioid receptors and the interacting mechanisms that induce physiologic changes as those needed to express sexual behaviour.

REFERENCES

Brooks, A.N., G.E. Lamming and N.B. Haynes, 1986 Endogenous opioid peptides and the control of gonadotropin secretion. Res. Vet. Sci., 41: 285-299.

Chemineau, P., G.D.J. Martin Suamande and E. Normant, 1988. Seasonal and hormonal control of pulsatile LH secretion in the dairy goat (Capra hircus). J. Reprod. Fert., 83: 91-97.

Cosgrove, J.R., F. Rensis and G.R. Foxcroft, 1993. Opiodergic pathways in animal reproduction: Their role and effects of their pharmacological control. Anim Reprod Sci., 33: 373- 392.

Currie, W.D., S.J. Cook and N.C. Rawlings, 1991. LH secretion in ovariectomized ewes: Effects of morphine and ovarian steroid interactions with naloxone during the breeding season and anestrum. Can. J. Anim. Sci., 71, 333-342.

Currie, W.D., I.B.J.K. Joseph and N.C. Rawlings, 1991. Morphine, naloxone and the gonadotropin surge in ewes. J. Reprod. Fert., 92: 407-414.

Currie, W.D. and N.C. Rawlings, 1989. Fluctuation in responsiveness of LH and lack of responsiveness of FSH to prolonged infusion of morphine and naloxone in the ewe. J. Reprod Fert., 86: 359-366.

Delgadillo, J.A., G. Fitz-Rodriguez, J. Duarte, F.G. Veliz, E. Carrillo, J.A. Flores, J. Vielma, H. Hernandez, Malpaux and Benoit, 2004. Management of photoperiod to control caprine reproduction in the subtropics. Reprod. Fertil. Dev., 16: 471-478.

Edqvist, L.E. and G.H. Stabenfeldt, 1993. The hormones of the reproduction. In: King G, (Ed.), Reproduction in Domesticated Animals. Amsterdam: Elsevier Sci. Pub., pp: 55-74.

Fabre-Nys, C., P. Poindron and J.P. Signoret, 1993 Reproduuctive behaviour. In: King G, editor. Reproduction in domesticated animals. Amsterdam: Elsevier Sci. Pub., pp: 147-182.

Fuentes V.O., 1988. Effect of naloxone, nalbuphine, progesterone and pregnant mare´s serum gonadotrophin on the sexual behaviour of ewes. Vet. Rec., 124: 274-276.

Fuentes H.V.O., P.I. Fuentes and A. García, 1997. Chronic treatment with naloxone enhace libido in the male goat during anoestrus. Vet. Rec., 141: 52.

Fuentes, V.O., V.M. Sanchez, H. Gonzalez, P.I. Fuentes, A. Garcia and R. Rosiles, 1998. La función endócrina del testículo en el carnero criollo mexicano durante las diferentes épocas del año y su control opioidergico durante el anestro. J. Vet. Med. A., 44: 259-263.

Fuentes, V.O., H. Gonzalez, V. Sanchez, A. García and P. Fuentes, 1998. The effect of naloxone on the duration of oestrus ovulation rate and oestradiol 17â in crossbred ewes with induced oestrus during seasonal anoestrus. Small Rum Res., 29: 89-92.

Fuentes, V.O., P.I. Fuentes, G. Gonzalez-Ruiz and V.M. Sanchez-Parra, 2006. The effect of bromocryptine on plasma prolactin concentration and ovulation rate in ewe breeds with different fecun dite rates. Small Rumin. Res., 63: 199-203.

George, S.R., T. Fan, Z. Xie, R. Tse, V. Tam, G. Varghese and B.F. O'Dowd, 2000. Oligomerization of mu-and delta-opioid receptors. Generation of novel functional properties. J. Biol. Chem., 275: 26128-26135.

Grossman, A., 1988. Opioid peptides and neuroendocrine function in man. In: Imura H. editor. Tokyo: Japan Scientific Societies Press, pp: 93-103.

Goodman, R.L., D.B. Parfitt, N.P. Evans, G.E. Dahl and F.J. Karsch, 2006. Endogenous opioid peptides control the amplitude and shape of gonadotropin-releasing hormone pulses in the ewe. Theriogenol, 138: 2412-2420.

Malven, P.V., 1995. Role of endogenous opioids for regulation of the oestrous cycle in sheep an cattle. Reprod Domest Anim., 30: 183-187.

Mori, Y., 1989. Central Integration of Photoperiodicity for Gonadotropin Release in Ruminants. In: Dyer EG, Bicknell RJ. (Eds.), Brain opioid systems in reproduction. Oxford: Oxford Sci. Pub., pp: 93-118.

Pasternak, G.W., 2001. Incomplete cross tolerance and multiple mu opioid peptide receptors. Trends Pharmacol. Sci., 22: 67-70.

Powell, K.J., N.S. Abul-Husn, A. Jhamandas, M.C. Olmstead, R.J. Beninger and K. Jhamandas, 2002. Paradoxical Effects of the Opioid Antagonist Naltrexone on Morphine Analgesia, Tolerance and Reward in Rats. Pharmacol. Exp. Ther., 300: 588-596.

Rekwot, P.I., D. Ogwn, E.O. Oyedipe and V.O. Sekoni, 2001. The role of pheromones and biostimulation in amimal reproduction. Anim. Rep. Sci., 65: 157-170.

Schall, R.E., F.J.P. Ebling, F.J. Karsch and D.L. Foster, 1991. Postpubertal maturation of endogenous opioid regulation of luteinizing hormone secretion in the female sheep. Biol. Reprod, 44: 760-768.