GLP-1 REGULATES THE REPRODUCTIVE FUNCTION AND SYNCHRONIZES THE ONSET OF THE PUBERTY IN FEMALE RATS

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INTRODUCTION

GLP-1 is an intestinal peptide with anorexigenic and insulinotropic effects. Exendin-4 (Ex4) is a GLP-1R agonist with longer half-life than GLP-1 used in the treatment of DM2. Previous studies from our group revealed the presence of GLP-1R in the three main levels of the gonadotropic axis (hypothalamus-pituitary-gonads). In addition, GLP-1 but not Ex-4 increases the amplitude of the pre-ovulatory surge.

OBJECTIVES: To evaluate the effects of native GLP-1(7-36)-NH2 (GLP-1) and Ex4 in the function of the reproductive system in adulthood and onset of puberty in female rats.

METHODS

STUDIES IN ADULTHOOD: Adult female rats were *icv* injected with a single dose of GLP-1/Ex4 (1 nmol) or vehicle (Veh) in the morning of proestrous. Blood samples were taken by jugular venipuncture at different stages of the subsequent estrous cycle: Proestrous (**P**), Estrous (**E**), Diestrous-1 (**D1**) and Diestrous-2 (**D2**) for hormonal determinations (LH, FSH, Estradiol, Progesterone). Ovaries were isolated for histological studies: the number of mature follicles were determined in slides from ovaries at afternoon of proestrous. Slides from ovaries at D1 were used to determine ovulation rate (Nº corpus luteum/rat).
Another group of females was housed with males for mating that confirmed by sperm-positive vaginal plug. The dams were sacrificed by decapitation after delivery, and number of undelivered but implanted fetuses and neonates born were counted and weighed.

STUDIES IN PREPUBESCENTS: Prepubertal rats were treated *icv* every 12h with GLP-1 (1nmol) or Ex4 (0.5nmol) for 8 days (27-35 d.). Individual monitoring of body weight, food intake and vaginal opening (AV as an early indicator of the onset of puberty) was performed. Serial blood samples for subsequent determination of the LH levels were collected. After treatment, the animals were sacrificed and their ovaries and uterus were weighed.

RESULTS

ADULTHOOD

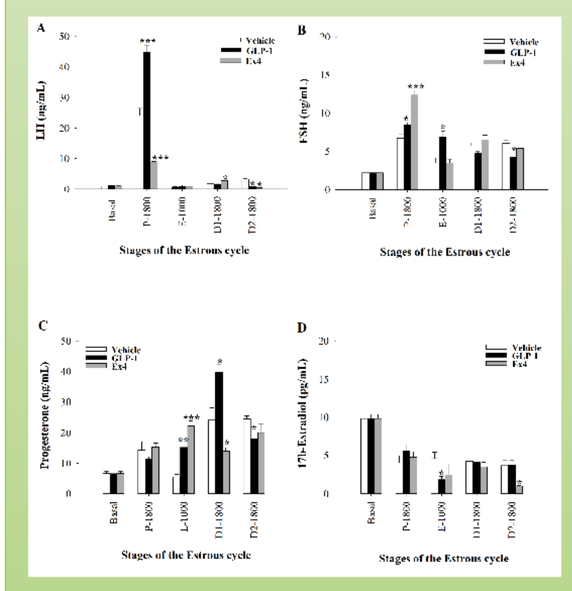

FIGURE 1. EFFECT OF GLP-1 OR EXENDIN-4 ADMINISTRATION *(icv.)* IN GONADOTROPIN AND GONADAL STEROID LEVELS OF ADULT FEMALE RATS.

Central injection of 1nmol of GLP-1 induced a significant increase in serum LH levels vs. vehicle group, at 18 p.m. of proestrous. Afterwards, LH levels decreased reaching nadir values in D2. Conversely, Ex4 significantly decreased LH levels, inducing a partial blockade of the LH pre-ovulatory surge (**Fig. 1A**).
A single dose of GLP-1 produced a significant increase in serum FSH levels vs. vehicle group, at 10 a.m. of estrous; but a significant reduction at D2. Ex4 nearly doubled FSH levels vs. control group, at proestrous afternoon. (**Fig. 1B**).
GLP-1 produced a significant increase in Progesterone secretion at estrus and D1 (coinciding with the corpus luteum development). Moreover, Ex4 also induced a significant increase in Progesterone serum levels vs. vehicle group, at estrous (**Fig. 1C**).
Acute treatment with GLP-1 produced a significant decrease in serum 17β-Estradiol levels vs. control group, at estrus. Ex4 decreased serum 17β-Estradiol levels at D2. (**Fig. 1D**).
*Mean±SEM of 10-12 independent determinations per group. Kruskal-Wallis Test * (p <0.05) ** (p< 0.01), *** (p< 0.001).*

Table1. Number of mature follicles and corpora lutea in the ovaries of female rats treated with vehicle, GLP-1 or Ex4.

Ovaries from rats administered with a single *icv* dose of GLP-1 showed a significant increase in the number of mature follicles, but in a lesser magnitude than those observed in ovaries from superovulated rats. On the contrary, Exendin-4 did not modify the number of mature follicles vs. control group.
GLP-1 significantly increased the ovulation rate respect to control group, with values slightly lower than those observed in ovaries from superovulated rats. On the contrary, Ex-4 did not affect the ovulation rate.
*Mean±SEM of 10-12 independent determinations per group. Kruskal-Wallis Test ** (p< 0.01), *** (p< 0.001).*

GROUP	Nº MATURE FOLLICLES. MEAN ± S.E.M	Nº CORPUS LUTEUM. MEAN ± S.E.M.
VEHICLE	13.75 ± 0.48	39.67 ± 0.33
SUPEROVULATED	53.50 ± 5.12 ***	66.30 ± 2.03 ***
GLP-1	31.75 ± 1.03 **	53.67 ± 1.20 **
Ex4	11.00 ± 0.57	33.67± 2.03

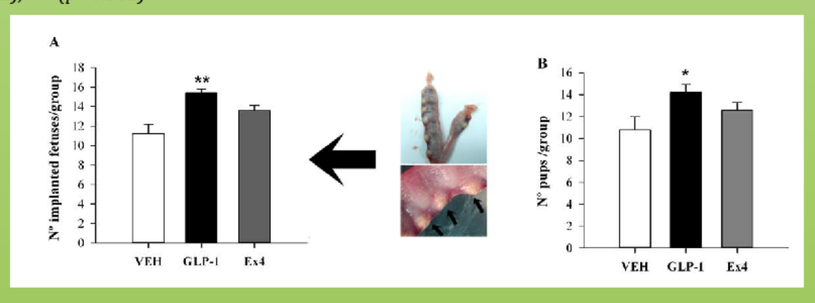

FIGURE 2. EFFECTS OF CENTRAL ADMINISTRATION OF GLP-1 AND EX4 IN LITTER SIZE AND NUMBER OF FETUSES.

Females treated with GLP-1 prior to pregnancy had a higher number of offspring (**Fig. 2A**) and implanted fetuses (**Fig.2B**) compared to the control group. However, Ex4 treatment did not produce change in litter size nor in the number of implanted fetuses respecto to controls.

*Mean ± SEM 10-12 independent determinations per group. Kruskal-Wallis Test * (p <0.05) ** (p <0.01)*

PUBERTY

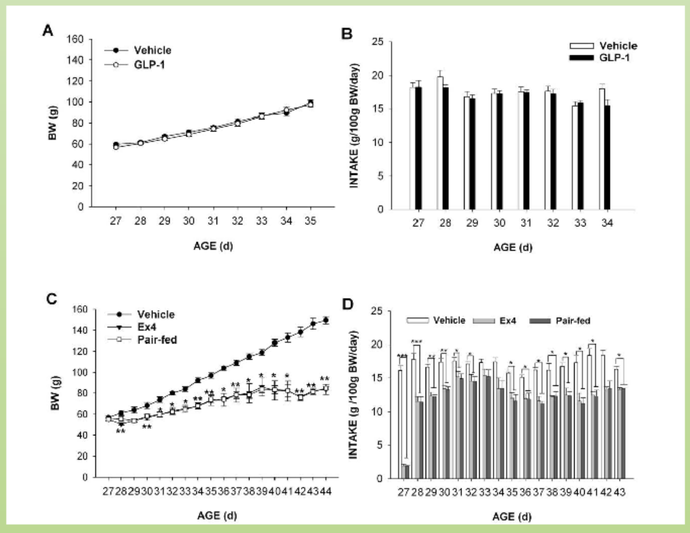

FIGURE 3. EFFECT OF CENTRAL *(icv.)*ADMINISTRATION OF GLP-1 AND EXENDIN-4 IN FOOD INTAKE AND BODY WEIGHT OF PREPUBERTAL FEMALE RATS.

Prolonged administration of 1 nmol/12h GLP-1 (icv.) did not change body weight or daily food intake during the experimental period respect to the control group (**Fig. 3A and 3B**). By contrast, administration of 0.5 nmol/12h of Exendin-4 (icv.) produced a significant decrease in body weight and daily food intake vs control group (**Fig. 3C and 3D**). Because of the effect of Ex4 in intake, a group of prepubertal female rats were allowed access to just the matched amounts of food by the Ex4-treated group (pair-fed group), to distinguish the influence of energy reduction and the direct effect of the studied peptides (**Fig. 3C and 3D**).
*Mean±SEM of 10-12 independent determinations per group. Kruskal-Wallis Test * (p <0.05) ** (p <0.01), *** (p <0.001).*

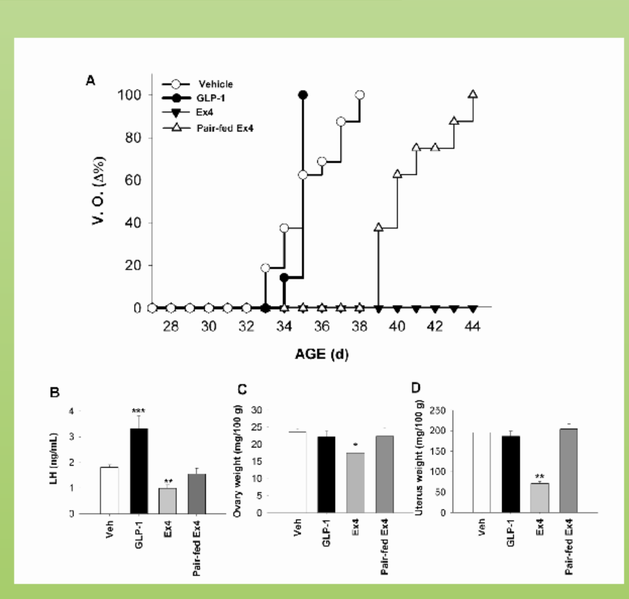

FIGURE 4. INDICES OF PUBERTAL MATURATION RECORDED IN FEMALE RATS CHRONICALLY EXPOSED TO GLP-1 OR EX4.

All animals treated with GLP-1 reached vaginal opening at day 35 of postnatal development (average 34.87 ± 0.35d, VO), while the control group showed VO ranging between days 33-37 (mean age 35.25 ± 1.73d of postnatal life). The pair-fed group showed delayed puberty onset (VO: 40.33 ± 1.51d) vs. control group. Ex4 treated animal failed to enter the puberty showing no vaginal opening in any case (**Fig. 4A**). VO data are expressed as a percentage of the total number of animals for each experimental group.
Prolonged administration of GLP-1 increased LH levels (**Fig. 4B**), although not modifying the weight of uterus and ovaries respect to controls (**Fig. 4C and 4D**). However, Ex4 treatment significantly decreased the levels of LH and weight of ovaries and uterus respect to the control group, justifying a blockade in puberty development. While the pair-fed group did not show any change in LH levels, ovary and uterus weight, respect to the control group.

*Mean±SEM 10-12 independent determinations per group. Kruskal-Wallis Test * (p <0.05) ** (p <0.01), *** (p <0.001).*

CONCLUSIONS

GLP-1 increases the preovulatory surge of gonadotropins and ovulation rate in adulthood and synchronize the onset of puberty. These effects were not reproduced by Ex4.