

Effects of Hydroxyprogesterone Caproate on the Weights of Developing Adrenal Glands in Albino Rats

JAVAID IQBAL, MOHAMMAD SUHAIL, ANEES FATIMA

ABSTRACT

Background: The wide use of hydroxyprogesterone caproate in the field of gynaecology and obstetrics necessitates considering its safety not only on the gonads of the fetuses but because of having a common metabolic background, on the developing adrenal glands.

Aim: To determine the effects of hydroxyprogesterone caproate on the developing adrenal glands of albino rats.

Methods: This experimental study was conducted at Department of Anatomy, Shaikh Zayed Federal Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-Azam Campus, University of the Punjab from March 2010 to July 2010. Twelve adult female rats weighing 250-300 grams and three adult male rats weighing 350-450 grams of Wistar strain were used in this experiment. Two experimental groups of female albino rats received hydroxyprogesterone caproate in doses of 10 and 25 mg per kg body weights. After parturition, the offsprings were sacrificed on 7th day. Their adrenal glands were dissected out and studied for gross features. The mean paired weight of adrenal glands in experimental groups was significantly increased as compared to control.

Results: The results showed that significant increase in weights of paired adrenal glands and relative tissue weight indices in both experimental groups as compared to the control group ($P < 0.05$).

Conclusion: The study showed that hydroxyprogesterone caproate given in a critical period of development of adrenal glands in a rat pup may cause accelerated maturity of the gland evident by the increase in their weights.

Keywords: Hydroxyprogesterone caproate, adrenal gland, albino rats

INTRODUCTION

This study was carried out to observe the effects of hydroxyprogesterone caproate on the developing adrenal gland of albino rat. Hydroxyprogesterone caproate has a wide and diverse range of uses. It is commonly used in practice of gynaecology and obstetrics to prevent premature labor, abortions both threatened and habitual as well as in cases of infertility due to insufficiency of corpus luteum.^{1,2} It has been well investigated in the reproductive organs. However the present study was designed to probe its effect on the adrenal glands especially during its critical period of development. Due to a common metabolic link of hormonal pathway through which it is related to the gonads, it was expected that the hydroxyprogesterone caproate might also affect the adrenal gland. Since the end of second week of development is a critical period for development of adrenal gland in rats, therefore this period was chosen for this study³.

MATERIALS AND METHODS

This experimental study was conducted at Department of Anatomy, Shaikh Zayed Federal Postgraduate Medical Institute, Lahore in collaboration with Department of Zoology, Quaid-e-

Azam Campus, University of the Punjab from March 2010 to July 2010. Twelve adult female rats weighing 250-300 grams and three adult male rats weighing 350-450 grams of Wistar strain were used in this experiment. After an acclimatization period of two weeks, the rats were weighed and an average weight gain of 25 gm/rat was noted. Males were determined for their sexual maturity by the aggressive behavior and free hanging of the testes in the scrotum. The female rats were then divided randomly into three groups: group A (Control), group B (Experimental) and group C (Experimental). Each group comprised 4 female rats.

The well known smear method was employed for the determination of estrous. In addition to vaginal smear, some other reliable vaginal as well as behavioral criteria were also used.

Three estrous females were placed in a cage with one male for overnight and all the female rats were allowed to conceive. The pregnancies were confirmed by the examination of vaginal plug. The females determined positive for having been mated were isolated. That marked day "0" of gestation.

The three groups of female rats were separated and were properly weighed, marked and placed in their respective cages and same marks were pasted on the cages.

Department of Anatomy, Shaikh Zayed Postgraduate Medical Institute, Lahore
Correspondence to Dr. Javaid Iqbal Email: drjsyed@hotmail.com

Group A (Control): All the four pregnant rats of this group were allowed to complete the pregnancy without exposure to hydroxyprogesterone caproate.

Group B: It contained four pregnant rats and they were given hydroxyprogesterone caproate 10mg/kg body weight intraperitoneally on days 14 and 15 of gestation.

Group C: It contained four pregnant rats and they were given hydroxyprogesterone caproate 25 mg/kg body weight intraperitoneally on days 14 and 15 of gestation. After the parturition of Group A, B, and C twenty offsprings from each group were selected at random for further procedure. The male offsprings were subgrouped as groups A₁, B₁ and C₁ respectively while the female offsprings were subgrouped as groups A₂, B₂ and C₂ respectively. They were placed in separate cages, which were properly labelled. On day 7 all the rats were euthanized with pentobarbital intraperitoneally with dose of 200 mg/kg^{4,5}. Each rat was weighed before dissection and following experimental procedure was done on these pups.

Gross appearance of the adrenal glands: The shape, position, color and weights of adrenal glands were recorded just after the dissection. This was calculated by the following formula:

$$RTWI = \frac{\text{Mean weight of both adrenal glands (micro grams)}}{\text{Mean body weight}} \times 100$$

Statistical analysis: The weights of the adrenal glands and relative tissue weight index were analyzed statistically by Analysis of Variance (ANOVA), using SPSS version 15. The P value less than 0.05 being significant for all analysis.

RESULTS

The adrenal glands appeared normal on gross examination with magnifying glass. These were present in their normal position occupying the superior poles of both kidneys, yellowish in color and triangular in shape. No apparent gross abnormality was observed in any control and experimental groups.

The results concerning the weights of the paired adrenal glands showed that the mean weight in group A in both sub groups A₁ and A₂ was 0.001µg (± 0.0004). The mean weight of paired adrenal glands in group B₁ was 0.002±0.004 µg and 0.0019±0.0007 µg in B₂. Group C pups showed mean weight of .0021µg in both subgroups C₁ and C₂ (±0.0004). The comparison of adrenal gland weights was statistically insignificant among pups of A₁ and A₂ (P>0.05) [Table 1]. Same pattern followed the comparison of both genders in experimental groups B₁ vs B₂ and C₁ Vs C₂ with no significant sex differences. However comparing the mean paired adrenal weights

of different groups showed that the weight in experimental groups B₁ and B₂ Vs C₁ and C₂ showed significant increase as compared to groups A₁ and A₂ (P<0.001) [Table 2]. No significant difference was noted among the weights of groups B₁ and B₂ Vs C₁ and C₂.

Relative Tissue Weight Index (RTWI): The mean relative tissue weight indices of both A₁ and A₂ pups in control group A was calculated to be 0.017 in A₁ (±004) and 0.019 in A₂ (±007). The mean RTWI in experimental group B was calculated to be 0.036 (±0.008) in B₁ and .040 (±0.011) in B₂. Similarly the mean RTWI for experimental group C was calculated to be 0.043 in C₁ (±0.007) and 0.044 (±0.008) in C₂ (Table 3). The mean relative tissue weight indices of group B (B₁ and B₂) and group C (C₁ and C₂) showed statistically significant increase when compared with control group A (A₁ and A₂) (P value <0.001) [Table 4]. While the increase in RTWI in group C₁ and C₂ as compared to group B₁ and B₂ was insignificant (P>0.05). With regards the sex of the pups, there was no statistically significant difference in RTWI among control and experimental pups (P>0.05)

Table 1: Weight of paired adrenal gland of pups of albino rats- control and experimental groups (µg)

Groups	Mean	SD	Minimum	Maximum
Male (A1)	0.0010	±0.0004	0.0005	0.0018
Females (A2)	0.0010	±0.0004	0.0006	0.0020
Male (B1)	0.0020	±0.0004	0.0016	0.0028
Females (B2)	0.0019	±0.0007	0.0010	0.0032
Male (C1)	0.0021	±0.0004	0.0016	0.0030
Females (C2)	0.0021	±0.0004	0.0016	0.0030

ANOVA

Source	Sum of squares	Df	Mean square	F	P value
Group	0.0000140	2	0.0000069765	29.8117	0.001*
Gender	0.0000000	1	0.0000000015	0.0064	0.936†
Group vs Gender	0.0000000	2	0.0000000105	0.4449	0.956†
Error	0.0000126	54	0.0000002340		
Total	0.0000266	59			

SD = Standard Deviation F = f-test (Ratio of variances)

Df = Degree of freedom †= Not significant (P>0.05)

* = Significant (P=< 0.001)

Table 2: Multiple comparisons of weight of paired adrenal glands of pups of albino rats-control and experimental groups

Groups	Groups	Mean difference	Std. Error	P value
Group A1 A2	Group B1 B2	-0.000975	.0001530	0.000*
	Group C1 C2	-0.001065	.0001530	0.000*
Group B1 B2	Group C1 C2	-0.000090	.0001530	0.559†

*=Significant (P<0.001) †=Not significant (P>0.05)

Table 3: Relative tissue weight index (RTWI) of pups of albino rats control and experimental groups

	Groups	Mean	SD	Minimum
Male (A1)	0.017	±0.004	0.012	0.025
Females (A2)	0.019	±0.007	0.013	0.031
Male (B1)	0.036	±0.008	0.027	0.054
Females (B2)	0.040	±0.011	0.026	0.058
Male (C1)	0.043	±0.007	0.032	0.055
Females (C2)	0.044	±0.008	0.032	0.060

ANOVA

Source	Sum of squares	Df	Mean square	F	P value
Group	0.006941	2	0.003471	60.61	0.001*
Gender	0.000079	1	0.000079	1.39	0.244†
Group vs Gender	0.000008	2	0.000004	0.07	0.929†
Error	0.003092	54	0.000057		
Total	0.010121	59			

SD = Standard Deviation F = f-test (Ratio of variances)
 Df = Degree of freedom †=Not significant (P>0.05)
 *=Significant (P=< 0.001)

Table 4: Multiple comparisons of relative tissue weight index (RTWI) of pups of albino rats – control and experimental groups

Groups	Groups	Mean difference	Std. Error	P value
Group A1 A2	Group B1 B2	.0197	.002393	.001*
	Group C1 C2	.02500	.002393	.001*
Group B1 B2	Group C1 C2	-.00530	.002393	.031†

*=Significant (P<0.001) †=Not significant (P>0.05)

DISCUSSION

In this study, pregnant females of control group did not receive any drug and the other two experimental groups were given hydroxyprogesterone caproate in increasing doses. After completion of gestation, the pups of respective groups were evaluated by various gross features. The adrenal glands also were normal in appearance and sites in all the three groups. There was however significant increase in the weights of paired adrenal glands of both experimental groups B1, B2 and C1, C2 in comparison with control groups A1, A2 (P<0.001). This increase in the paired adrenal weights in the groups exposed to hydroxylprogesterone caproate may indicate the hypertrophic state of the gland itself in accordance to studies by Pellegrini⁶ and Bauer.⁷ There was however no significant difference in the weights of adrenal glands of male and female pups in any of the three groups (P>0.05).

The mean relative tissue weight index also showed significant increase in experimental groups as compared to the control groups (P<0.001). However there was no difference in relative weights among genders of any of the groups (P>0.05). Even in control groups A1, A2 the relative weights for

adrenal glands between male and female pups did not show any significant difference. This was in contrast to a study conducted by Proshaska.⁸ He while studying effects of copper depleted diets found out that in rats of copper depleted as well as copper adequate groups the relative weights of adrenal glands of rats were influenced by gender with female weights higher than male weights. Being a steroidal hormone, hydroxyprogesterone caproate was expected to produce atrophy of the adrenal gland by the activation of HPA axis indicated by decrease in weight of adrenal glands but instead there was hypertrophy of the gland This finding was collaborated by another study by Masayuki Nakano⁹ undertaken in 1981 which showed that betamethasone when given in utero to rat fetuses produced adrenal hypertrophy. It crossed the placental barrier and exerted its hypertrophic effect on the gland. As HPC has also been documented in crossing the placental barrier, it may also produce the same effect as that of exogenous betamethasone. Hydroxyprogesterone caproate has been documented to be a weak stimulator of HPA axis, therefore this could also be the reason that it did not exert an atrophic effect instead increasing the weights of the adrenal gland¹⁰.

REFERENCES

1. Alpha Hydroxyprogesterone Caproate for prevention of preterm birth overview of FDA background document. 2006
2. Pharmaoffshore.com
3. Phoebe Dewing, Saunders T. Ching, Yao-Hua Zhang, Bing-Ling Huang et al. Midkine is expressed early in rat fetal adrenal development. Mol gen Met 2000; 71(4): 616-22.
4. Ustin H, AkgulKT, Ayyildiz A, Yagmurdur H, Nuhoglu B et al. Effect of phosphodiesterase 5 inhibitors on apoptosis and nitric oxide synthetase in testis torsion: an experimental study. Pediatr SurgInt 2008;24:205.
5. AVMA guidelines on Euthanasia
6. Pelligrini A, Grieco M, Materazzi G, Gesi M, RiccardiMP. Stress induced morphological and functional changes in adrenal cortex, testis and major salivary glands. Histochem J 1998;30:695-701.
7. Bauer MS, Perks P, Lightman SL, Shanks N. Restraint stress is associated with glucocorticoidimmunoregulation. Phy Behaviour 2011;73:525-32.
8. Proshaka JR, Brokate B. Dietary copper deficiency alters protein levels of rat dopamine β-monooxygenase and tyrosine monooxygenase. Exp Biomed 2001; 226: 199 -207.
9. Masayuki Nakano, Masanori Nishiuchi, Masaharu Takeuchi, Hideo Yamada. Correlation between metabolism of betamethasone 17,21-dipropionate and adrenal hypertrophy in rat fetuses. Steroids 1981;37(5):511-25.
10. Mughal IA, Qureshi SA, Tahir MS. Some histological observations on post natal growth of rat adrenal gland with advancing age 9A HRLM Study). Int J Agri Bio 2004;6(4):413-17.