Original article

THE IMPACT OF HYDROXYPROGESTERONE CAPROATE ON ADRENAL CORTEX THICKNESS IN DEVELOPING RATS

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ABSTRACT

Background: Hydroxyprogesterone caproate, a drug used commonly in Gynaecology and Obstetrics, has been shown to increase the weight of developing rat adrenal glands. This research was undertaken to observe changes in the various zones of the adrenal cortex with an increase in the thickness of the adrenal cortex.

Material and Methods: Hydroxyprogesterone caproate was administered to two experimental groups at doses of 10mg and 25mg per kg body weight intraperitoneally, while the third group was the control group and didn't receive any hydroxyprogesterone caproate. On seventh day following delivery, the offspring were sacrificed. The adrenal glands were dissected and after proper fixation and staining, the thicknesses of various zones of the adrenal cortex were noted.

Results: It was observed that the mean thickness of the adrenal cortex was increased in experimental groups as compared to the control group.

Conclusion: The research revealed that hydroxyprogesterone caproate impacted rat pups' adrenal cortex by increasing its thickness and accelerating its maturity when used in a critical phase of the development of the adrenal gland.

Key Words: Adrenal cortex, Rats, Corpus luteum

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INTRODUCTION

Hydroxyprogesterone caproate, a drug most commonly used in Gynaecology and obstetrical practice, has been well-researched on the reproductive organs in both males and females.[1] Its vast therapeutic applications include the prevention of premature labor, both threatened and habitual abortions, and cases of infertility related to corpus luteum insufficiency[1,2] necessitate that its harmful effects if any should be well documented.

As the second week of development is the most sensitive period in formation of the adrenal gland in rats, this period was chosen in the current study to examine its impact on the adrenal gland.[3]

The previous studies have shown that this drug increases the weight of the adrenal gland[4], so it is expected to further link this effect to the overall thickness of the adrenal cortex of the gland.

MATERIAL AND METHODS

From March to July 2010, this experimental investigation was carried out at the Department of Anatomy, Shaikh Zayed PGMI, Lahore, in partnership with the Department of Zoology, University of the Punjab (Quaid-e-Azam Campus).

In this experiment, Wistar strain adult rats weighing a mean of 250–300 grams for each of the twelve females and 350–450 grams for each of the three males were employed. The rats were weighed two weeks after the acclimatization phase and weight gain of 25 gm/rat was recorded. Male rats with aggressive behaviour and the testes freely

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hanging in the scrotum were used to assess their sexual development.

Afterward, the female rats were separated into Group A (Control), Group B (Experimental) & Group C (Experimental) based on a random selection with 4 rats each, based on a random selection.

In addition to vaginal smear, the smear method was utilized to determine estrous. To confirm estrus, behavioral changes were also taken into consideration.

All the female rats were allowed to procreate after being placed in a cage containing three estrous females and one male for one night. Vaginal plug appearance was regarded as a sign of pregnancy. The female rats that had been found to have been mated were separated. That was the first day of gestation.

After correctly weighing and marking, the female rats were divided and placed in their corresponding cages.

The study had three groups. Group A was controlled group with four pregnant rats were permitted to finish their pregnancies without being exposed to hydroxyprogesterone caproate.

Group B was experimental group which had four pregnant rats. On days 14 and 15 of their gestation, they received an intraperitoneal injection of hydroxyprogesterone caproate at a dose of 10 mg/kg of body weight.

Group C was experimental group which had on day 14 and 15, four pregnant rats from this group received intraperitoneal injections of 25 mg/kg body weight of hydroxyprogesterone caproate.

Twenty offspring from each group were randomly chosen for further procedure after delivery of the pups.. The male offspring were divided into groups A1, B1, and C1, while the female offspring were divided into groups A2, B2, and C2. They were put in distinct cages with the appropriate labels. On the seventh day all the rats were euthanized with 200 mg/kg of pentobarbital intraperitoneally.[5, 6]

The adrenal glands were fixed in 10% formalin. The specimens were preserved and embedded in paraffin wax. With the help of a rotary microtome, 5µm sections were cut and stained with haematoxylin and eosin (H&E) stain.

The adrenal glands were observed histologically for overall thickness of the adrenal cortex(µm) in addition to the thicknesses of its zones.

These measurements were taken with a correctly calibrated ocular micrometer.

At the end of the research experiment, quantitative data were analyzed by Analysis of Variance (ANOVA), using the statistical package of social sciences (SPSS) version 15.0. The obtained results were tabulated and compared with national and international published studies and finally, the conclusion was reached. A P-value less than 0.05 was deemed statistically significant for analysis.

RESULTS

The results of the experiment showed that the mean thickness of the adrenal cortex in the rat pups in A1 was 179.5µm (±12.9) and 182µm in A2 (±6.2). Similarly, the overall thickness of the adrenal cortex in group B1was 250.3 µm (±8.4) and 243.0 µm (± 16.2) in B2. In C1 it was calculated to be 334.3µm (± 12.4) and 332.0 µm (±7.5) in C2 (Table-1). This difference was calculated to be statistically significant ($P < 0.001$) while comparing A1, A2 with B1, B2 and C1, C2. Comparing the thickness of the adrenal cortex also revealed marked increase in C1, C2 compared to B1, B2 ($P < 0.001$, Table-1). No significant difference was noted as regards the two genders (Table-2).

This increase in cortical thickness was associated with an increase in the thickness of the individual zones of the adrenal cortex namely the zona glomerulus, zona fasciculate and the zona reticularis. However, the zona fasciculate displayed the most noticeable rise in thickness compared to other zones.

The zona glomerulosa showed a mean thickness of 13 to 13.5 (A1 and A2) with 23.8 and 21.3 in B1, B2 and 30 in C1 and C2 respectively. The zona fasciculate showed a mean thickness of 154 to 156 in A1, A2 and 204 to 205 in B1B2. The mean thickness of 278.5 and 276.5 was observed in C1 and C2. The results for zona reticularis were 12.5 and

12 in A1, A2 whereas in B1, B2 it was 23.8 and 21.8 while in C1, C2 the values were 25.8 and 25.5 (Fig1, 2)

Table-1. Thickness of the adrenal cortex of albino rat pupps in control & experimental groups (µM)

Group	Mean	S.D.	Min	Max
Male (A1)	179.5	±12.9	150.0	197.5
Females (A2)	182.0	±6.2	175.0	190.0
Male (B1)	250.3	±8.4	235.0	267.5
Females (B2)	243.0	±16.2	200.0	255.0
Male (C1)	334.3	±12.4	310.0	350.0
Females (C2)	332.0	±7.5	320.0	345.0

Table-2. Comparisons of the thickness of adrenal cortex in albino rat pupps (experimental and control groups)

Group	Group	Mean difference	SE	p-value
Group A1A2	Group B1B2	-65.87	3.525	< 0.001*
Group A1A2	Group C1C2	-152.38	3.525	< 0.001*
Group B1B2	Group C1C2	-86.50	3.525	< 0.001*

SE Std. Error
*Significant difference (P <0.001)

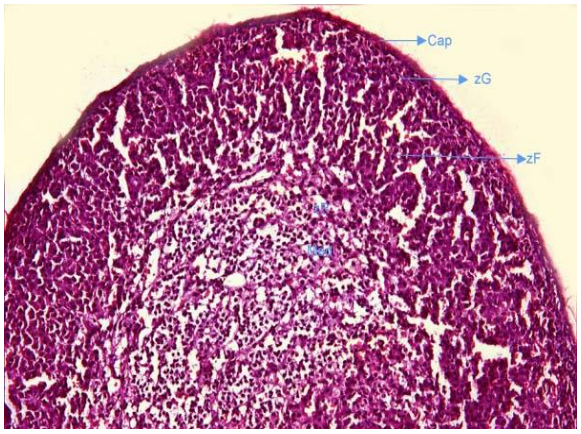

Figure-1: Photomicrograph showing adrenal cortex of rat pup of control groups A (H&E,5)

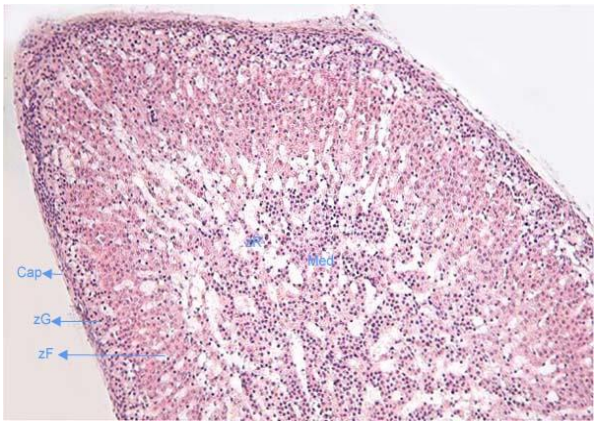

Figure-2: Photomicrograph showing adrenal gland with increased thickness in adrenal cortex of rat pup of experimental group B (H&E,5)

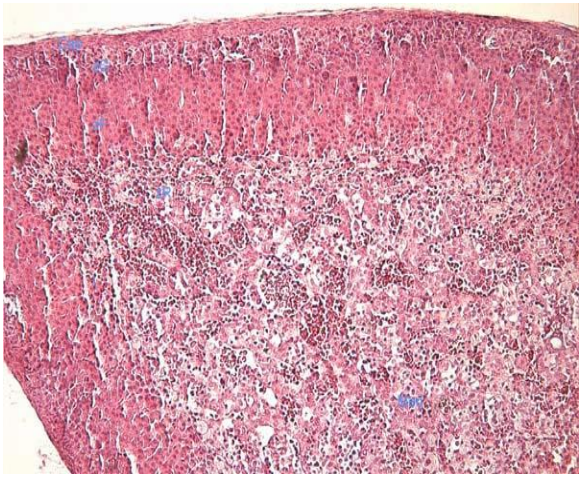

Figure-3: Photomicrograph showing adrenal gland with increased thickness in adrenal cortex of rat pup of experimental groups C (H&E,5)

DISCUSSION

The current study was meant to investigate its impact on the shape of the adrenal gland, particularly during its critical development time. The adrenal cortex and all three of its zones showed hypertrophy, which was evident by an increase in the thickness of each zone.[7] The zona glomerulosa showed an increased thickness and hypertrophy in experimental groups B1, B2 and C1C2 compared to control groups A1 and A2 (P<0.001). Further, the cells of this zone also showed an increase in mean size in experimental groups. There was no

difference in the thickness of zone and cell size regarding the genders in any of the groups (P>0.05). The zone also showed changes in its appearance as more organized patterns of cluster formation were evident in both experimental groups while this was only sporadic in the control groups.

The zona fasciculata, like the pattern of zona glomerulosa, also showed increased thickness and hypertrophy in pups exposed to hydroxyprogesterone caproate than in the control groups (P<0.001). The mean cell size of this zone also increased significantly in experimental groups (P<0.001). However, this zone showed more profound changes in the groups C1 and C2, who received the maximum dose of HPC compared to experimental groups B1 and B2 (P<0.001). Again, the zone appeared more organized in experimental groups, with cells showing increased vacuole formation.

The innermost zone of the adrenal cortex, i.e., the zona reticularis also responded to the exogenous hydroxyprogesterone caproate by an increase in thickness and the mean cell size (P<0.001). Additionally, it revealed that experimental pups had significantly more hypertrophy than pups from control groups (P<0.001). The zone which showed diffuse arrangement in the control groups showed better cord-like arrangement in the experimental groups.

Hydroxyprogesterone, a steroid, was supposed to produce atrophy of the gland using the HPA axis. Still, this effect of hypertrophy has been collaborated by Lolier showed that a steroid like betamethasone given to the rat fetuses produced hypertrophic effects to adrenal gland by crossing the placental barrier.[7,8] The same result as betamethasone was also produced by HPC as it crossed the placental barrier. This and the fact that HPC is a modest activator of the HPA axis failed to produce atrophy of the gland.[9] All the individual zones showed hypertrophy (increase in the width of their respective zones), but the difference was more marked in the zona fasciculate.[10] This was showed in a research work on stress and steroid-producing glands that showed an increase in thickness all three zones but the zona fasciculate which showed the most pronounced effect due to an increase in ACTH level.[11,12]

CONCLUSION

This study found that giving hydroxyprogesterone caproate to a rat pup during a critical stage of development of the adrenal gland may allow the gland to mature faster. This fact may be considered while giving hydroxyprogesterone caproate to prevent pre-term labor.

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Conflict of interest: None

AUTHOR'S CONTRIBUTION

JI: Manuscript writing and data collection
AS: Manuscript writing
HMD: Manuscript writing

REFERENCES

1. Forsberg JG, Iguchi T. Long-Term Effects of Perinatal Treatment with Sex Steroids and Related Substances on Reproductive Organs of Female Mice inToxicity of Hormones in Perinatal Life 2020 Apr 14 (pp. 60-80). CRC Press.
2. Mesiano SA, Peters GA, Amini P, Wilson RA, Tochtrop GP, van Den Akker F. Progestin therapy to prevent preterm birth: History and effectiveness of current strategies and development of novel approaches. Placenta. 2019 Apr 1;79:46-52. https://doi.org/10.1016/j.placenta.2019.01.018
3. Mesiano S. Endocrinology of human pregnancy and fetal-placental neuroendocrine development. InYen and Jaffe's reproductive endocrinology 2019 Jan 1 (pp. 256-284). Elsevier. https://doi.org/10.1016/B978-0-323-47912-7.00011-1.
4. Murphy CC, Cirillo PM, Krigbaum NY, Cohn BA. In utero exposure to 17α-hydroxyprogesterone caproate and risk of cancer in offspring. Am J Obstet Gynecol. 2022 Jan 1;226(1):132-e1. https://doi.org/10.1016/j.ajog.2021.10.035

5. Iqbal J, Suhail M, Fatima A. Effects of Hydroxyprogesterone Caproate on the Weights of Developing Adrenal Glands in Albino Rats. Group. 2015 Jan 1;1:A2.
6. Üstün H, Akgül KT, Ayyıldız A, Yağmurdur H, Nuhoğlu B, Karagüzel E, Öğüş E, Germiyanoğlu C. Effect of phospodiesterase 5 inhibitors on apoptosis and nitric oxide synthases in testis torsion: an experimental study. Pediatr Surg Int. 2008 Feb;24(2):205-11. https://doi.org/10.1007/s00383-007-2058-8.
7. Underwood W, Anthony R. AVMA guidelines for the euthanasia of animals: 2020 edition. Retrieved on March. 2020 Mar;2013(30):2020-1.
8. Lolier ML, Wagner CK. SUN-255 The Synthetic Progestin, 17-alpha-hydroxyprogesterone Caproate, Used in Human Pregnancy Alters Prefrontal Cortical Development in Rats. J Endocr Soc. 2020 Apr;4(Supplement_1):SUN-255. doi: 10.1210/jendso/bvaa046.
9. Christian MS, Brent RL, Calda P. Embryo–fetal toxicity signals for 17α-hydroxyprogesterone caproate in high-risk pregnancies: A review of the non-clinical literature for embryo–fetal toxicity with progestins. J Matern Fetal Neonatal Med. 2007 Jan 1;20(2):89-112. https://doi.org/10.1080/14767050601178758
10. Iguchi T, Sato T, Nakajima T, Miyagawa S, Takasugi N. New frontiers of developmental endocrinology opened by researchers connecting irreversible effects of sex hormones on developing organs. Differentiation. 2021 Mar 1;118:4-23. https://doi.org/10.1016/j.diff.2020.10.003.
11. Mughal IA, Qureshi AS, Tahir MS. Some histological observations on postnatal growth of rat adrenal gland with advancing age (AHRLM Study). Int. J. Agri. Biol. 2004;6(2):413-7.
12. Bianchi AB, Ruoti M. Prematurity: Evaluation of Fetal Well-Being and Delivery. In Perinatology 2022 (pp. 593-625). Springer, Cham. doi: 10.1007/978-3-030-83434-0_33