Journal of Steroid Biochemistry, 1970, Vol. 1, pp. 315–318. Pergamon Press. Printed in Great Britain

SUSTAINED RELEASE HORMONAL PREPARATIONS

X. UPTAKE OF 6-METHYL-17α-ACETOXY-4,6-PREGNADIENE-3, 20-DIONE RELEASED FROM POLYDIMETHYLSILOXANE IMPLANTS BY VARIOUS TISSUES OF MALE AND FEMALE RATS

I. ANGEE, C. C. CHANG, HARRY W. RUDEL and FRED A. KINCL
The Population Council, The Rockefeller University, New York, N.Y. 10021, U.S.A.

(*Received* 16 *January* 1970)

SUMMARY

Plasma levels and uptake by various tissues of 6-methyl-17α-acetoxy-4,6-pregnadiene-3,20-dione and metabolites released from polydimethylsiloxane implants were compared in female and male rats. In females circulating levels 4 days after implantation were 3120 ng/100 ml of plasma; in males the corresponding value was 2790 ng/100ml. The uptake by the hypothalamus, cerebellum, pituitary, adrenals, liver and body fat was higher in female animals than in male animals. Estrone treatment of males influenced uptake in the liver, adrenals and fat but not in the other organs.

INTRODUCTION

MEGESTROL acetate (6-methyl-17α-acetoxy-4,6-pregnadiene-3,20-dione) implanted subcutaneously in polydimethylsiloxane (PDS) capsules in male rats results in decreased uptake by various tissues as compared to oral administration[4]. The present communication describes circulating plasma levels and accumulation into various tissues of megestrol acetate absorbed from PDS capsules in female rats. A group of male animals pretreated with estrone was included for comparison.

MATERIALS AND METHODS

General conditions. Holtzman strain adult rats were used. The average weight of male animals was 425 g (range 320–570 g); of females 260 g (range 240–290 g). Two[6-^{3}H] megestrol acetate polydimethylsiloxane implants described previously were used[4]. The specific activity of the material was 2027 dpm/ng; average *in vitro* diffusion of each implant was 20·9 μg ± 1·2 (S.E.) per 24 hr. In two experiments males were pretreated with one PDS estrone (3-hydroxy-1,3,5(10)-estratrien-17-one) implant for 6 or 75 days, respectively. The implants were made by sealing dry, crystalline material in Silastic (Dow-Corning, Midland, Michigan) tubing No. 602-281. Implant length was 4 mm, wall thickness 0·8 mm, and average *in vitro* diffusion 3·6 ± 0·11 μg/24 hr (determined as described,[3]). Using slight ether anesthesia the implants were inserted subcutaneously in the dorsal region. All animals were kept in metabolic cages with water and food *ad libitum.* At autopsy blood was drawn from the posterior vena cava with a heparinized syringe, plasma was separated by centrifugation and frozen. Selected organs were removed, weighed and kept frozen until processed. In the brain 3 different areas were processed separately. A segment was removed between the optic chiasma and the third ventricle, 2–4 mm thick (hypothalamus); the average weight of tissue taken was between 51 and 85 mg. Next the cerebellum was separated (weight in females 220–290 mg; in males 210–336 mg). The rest of the brain weighed about 1400 mg. The preparation of the samples for the determination of radioactivity and other general conditions were as described[4]. The amount of megestrol acetate present in the various tissues (wet weight) was calculated on the basis of radioactivity present.

RESULTS AND DISCUSSION

Plasma levels in female rats and in estrone-treated males 4 days after implantation are compared in Table 1; the higher levels seen in female animals were not significant when the data were expressed as percentage of the *in vitro* dose and plasma volume (4%) was based on body weight.

Table 1. Plasma levels of megestrol acetate released from PDS implants

Days after implantation	3	4
Females: ng/100 ml plasma	2020 (2)*	3120 ± 202 (4)
Per cent dose†	0·98	1·61
Males: ng/100 ml plasma		2790 ± 94 (8)
(Estrone Per cent dose treated)		2·13

*( ) Number of animals used.
†See text.

There were no differences between males and females in excretion of radioactive materials (urine and feces) during the days after implantation. Total mean value after 4 days was 23·8 μg (range 20·5 to 27·1 μg) for females and 22·4 μg (range 13·6 to 36·2 μg) for males treated with estrone. Considerable material was found in the digestive tract. The value for females was 6·3 μg (range 2·9 to 9·5 μg); 65% of the radioactive material was present in the large and 35% in the small intestine. For males the mean was 8·6 μg (range 6·9 to 10·4 μg) and 71% was present in the large intestine.

The large amounts of material found in the digestive tract show that in white rats significant amounts of the drug enter into enterohepatic circulation. This raises the question of whether results seen in this particular species are indicative of possible effects in humans where 3 times as much of the metabolic products are excreted in the urine as in the feces [2].

Uptake of megestrol acetate by various organs is given in Table 2. Since there were no differences, data for both female groups were combined except for ovaries. For ovaries the mean value for day 3 was 55 ng/g and for day 4, 99 ng/g of tissue. In males estrone treatment apparently influenced uptake by the liver; the mean value was 153 ng/g ± 20·8 (S.E.) The relatively large standard error was due to the difference seen in the two groups. Mean value for animals treated for 6 days was 185 ng/g (range from 133 to 281 ng/g); in the group treated for 75 days the mean value was 120 ng/g (range 94 to 143 ng/g). Independent of the length of estrone treatment was the uptake in the brain. There were no differences in the three centers studied when the values were expressed in ng/g of tissue. However, differences in the actual amounts found in the three areas were considerable. For male animals the values were as follows (ng ± S.E.; $n = 8$): cerebrum, 37 ± 1·3; cerebellum, 8 ± 0·4; and hypothalamus 3 ± 0·3. For females ($n = 6$): cerebrum, 41 ± 1·8; cerebellum, 8 ± 0·8; and hypothalamus 6 ± 0·8.

The lack of specific uptake of megestrol acetate in the brain of both sexes deserves a comment. Seiki *et al.* [5] reported lack of specific progestrone binding centers in the brain of female rats. Our data show a uniform uptake of a synthetic progestational agent in the nervous system. Based on these two observations it may be speculated that the well known lack of antifertility activity of progestational agents in the rat can be explained by the absence of specific hypothalamic binding sites in this species.

There were differences in total amounts found in the sex organs. The values were: testes, 108 ± 8·0; epididymis, 36 ± 1·5; ventral prostate, 15 ± 2·3; seminal vesicles, 17 ± 1·6; for females: uterus, 14 ± 1·7; vagina, 4 ± 1·4; and ovaries, 6 ± 0·6.

Table 2. Uptake of megestrol acetate released from PDS implants by various organs in rats

Organ	ng found per g of tissue ±S.E. (range) Females*	Estrone treated males†
Liver	346 (246–446)‡	153 ± 20·8
Kidneys	38 (33–42)‡	32 ± 2·8
Cerebrum	33 ± 2·0	28 ± 0·9
Cerebellum	34 ± 1·4	26 ± 1·3
Hypothalamus	37 ± 2·6	27 ± 2·7
Ovaries	99 ± 7·1	
Uterus	32 ± 2·4	
Vagina	37 ± 1·6	
Fat	82 ± 10·3	31 ± 3·3
Adrenals	89 ± 8·4	52 ± 7·4
Pituitary	27 ± 3·3	18 ± 2·9
Carcass	13 ± 1·3	
Testes		30 ± 1·4
Epididymis		30 ± 1·2
Ventral prostate		31 ± 2·2
Seminal vesicles		30 ± 1·6

*$n = 6$.
†$n = 8$.
‡$n = 2$.

The total amount of radioactivity detected was expressed in terms of megestrol acetate although it was recognized that part of the material was metabolized. In one experiment we have partitioned total radioactivity between water and ether by combining extract from various animals. In plasma 80% of radioactivity was found in the aqueous phase and only 20% of the material was ether soluble. In contrast 72% of the material in the sex organs (ovaries, uterus and vagina combined) was extracted into ether and only 28% remained in water.

Comparison in the uptake of megestrol acetate and/or of its metabolites between female animals (this study) and males [4] is presented in Table 3. Signifi-

Table 3. Uptake of megestrol acetate by various organs in male and female rats

Tissue	ng per g of tissue Males (A)*	Females (B)	Ratio B/A
Cerebrum	29	33	1·14
Cerebellum	26	34	1·31†
Hypothalamus	25	37	1·48†
Pituitary	17	27	1·58†
Adrenals	33	89	2·70†
Liver	87	346	3·98
Kidneys	32	38	1·19
Fat	24	82	3·42†
Carcass	9	13	1·45

*Data from [4].
†$P = < 0{\cdot}05$.

cantly higher accumulation was seen in the female pituitary, hypothalamus, cerebellum, adrenals and fat. There was an apparent difference also in the liver uptake but because of the small number of observations (2 for females) this difference could not be statistically evaluated. Sex differences noted in the uptake of this material by various organs raise a question of the validity of using male animals in toxicity studies for compounds destined for exclusive use by women.

REFERENCES

1. C. C. Chang and F. A. Kincl: *Steroids* **12** (1968) 689.
2. J. M. Cooper and A. E. Kellie: *Steroids* **11** (1968) 133.
3. F. A. Kincl, G. Benagiano and I. Angee: *Steroids* **11** (1968) 673.
4. F. A. Kincl, I. Angee, C. C. Chang and H. W. Rudel: *Acta endocr (Kbh)* **64** (1970) 508.
5. K. Seiki, M. Miyamoto, A. Yamishita and M. Kotani: *J. Endocr.* **43** (1969) 129.