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ROLE OF THE HYPOTHALAMO-HYPOPHYSEAL AXIS IN NEONATAL ANDROGENISATION AND ITS POSTPUBERTAL EXPRESSION

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SUMMARY

Neonatal impregnation with gonadal steroids permanently affects protein synthesis and enzyme activity in a score of peripheral organs. Many, if not all, of these neonatal effects, however, do not come to expression in the absence of the pituitary or after disruption of the hypothalamo-hypophyseal axis. It may be hypothesized that during the neonatal period of differentiation gonadal hormones permanently change the production or secretion of known or unknown hypophyseal hormones.

At this point the question should be asked whether all neonatal effects of gonadal hormones are mediated by the same or by different hypophyseal factors? A survey of the pertinent literature reveals 6 clusters of proteins and enzymes whose concentration or activity in adulthood is jointly, but in a distinct way, regulated by pituitary and gonadal hormones. Proteins belonging to two of these clusters were studied longitudinally by radial immunodiffusion in hypophysectomized rats and in hypophysectomized rats with pituitary implants and this before and after treatment with CB 154. Prolactin seems to be the mediator of the effects of gonadal hormones on serum transcortin levels.

INTRODUCTION

Neonatal impregnation with gonadal steroids seems to influence a score of organs all over the body [1]. However, in adulthood most, if not all, of these neonatal effects of gonadal steroids disappear when the animals are hypophysectomized or after disruption of the hypothalamo-hypophyseal axis. This could mean that neonatal impregnation with gonadal steroids affects primarily the brain, i.e. the pituitary, the hypophysiotropic area or higher echelons, and that the other effects are secondary to this brain differentiation. At this point several questions can be asked. I.a. is one or more than one pituitary factor involved and how is the production of this hypophyseal factor(s) influenced by neonatal androgenisation?

The answer to these questions is obscured by the multitude of interrelations which exist between gonadal steroids, on the one hand, and the hypothalamo-hypophyseal axis on the other hand. Besides the effects which are abolished when the pituitary is removed or the hypothalamo-hypophyseal axis disrupted, many other effects of gonadal steroids are not abolished but modified in the absence of hypophyseal hormones or of their peripheral equivalents [2]. Furthermore, a survey of the literature (Table 1) indicates that there are several varieties of gonadal steroid effects which are abolished after hypophysectomy. Indeed, according to the 3 pairs of criteria outlined in the following paragraph, 8 clusters of serum proteins or enzyme activities can be delineated, proteins or enzymes whose levels or activities in adulthood are affected by gonadal steroids only when the hypothalamo-hypophyseal axis is intact.

1. As far as some protein concentrations or enzyme activities are concerned the effect of androgens is synergistic with the effect of the pituitary, while for other parameters the opposite is true. Similarly, estrogens affect some parameters in the same direction as the pituitary and the opposite is true for other proteins or enzymes.

2. Some of these protein concentrations or enzyme activities can only be influenced by estrogens, while others are affected by androgens as well as by estrogens [7, 8].

3. In most instances adult males have higher enzyme activities or higher protein concentrations than adult females (m > f), but in other instances the opposite is true (m < f).

Examples of 6 out of the 8 possible clusters showed up by analysis of the literature (Table 1). We have studied examples of two of these clusters.

METHODS

Uncoupling of the pituitary as a tool for distinguishing between two classes of pituitary hormones. Uncoupling of the pituitary, for instance, by removing the gland from the sella tursica and implanting it under the renal capsule, makes it possible to distinguish between two classes of pituitary hormones. The secretion of the first group increases by uncoupling, i.e. in the absence of hypothalamic inhibiting factors. The known examples of this group are prolactin and MSH. Uncoupling on the other hand, decreases dramatically the secretion of those pituitary hormones whose secretion requires the constant presence of hypothalamic liberating (releasing) factors.

Table 1. Clusters of protein concentrations or of enzyme activities regulated by pituitary hormone(s) and influenced indirectly by gonadal steroids

	Influenced by androgens or by androgens and estrogens		Only influenced by estrogens	
	Androgen effect parallels the effect of the pituitary	Androgens antagonize the effect of the pituitary	Estrogens antagonize the effect of the pituitary	Estrogen effect parallels the effect of the pituitary
Intact males have higher values than females	L α_{2U} [4]	L 2α hydrox, mi [6] L 3β hsd, mi [5,6] L 6β hydrox, mi [6] L 7β hydrox, mi [6] L 16α hydrox, mi [6] L 18 hydrox, mi [6]	L 17β hsd, c [7]	L 5β red. c [7] K 11β hsd, mi [8]
m > f		L 20α hsd, mi [6]		
Intact males have lower values than females m < f		L, transcortin [2] L, 5α red, mi [3,5,6] L, 7α hydrox, mi [6,7] K, 17β hsd, c [7]		K 3α NADPH, c [8]

K: kidney, L: liver, mi: microsomal enzyme; c: cytosolic enzyme; hydrox: steroid hydroxylase; hsd: hydroxysteroid dehydrogenase; red: steroid reductase; 3α NADPH: NADPH-dependent 3α hsd; 3α NADH: NADH-dependent 3α hsd.

Examples of the latter group are ACTH, TSH, LH and FSH, and perhaps GH.

Denef [5] has used this approach and could show that while hypophysectomy (as does testosterone) increased the activity of the 3β-hydroxysteroid dehydrogenase in gonadectomized females, implantation of a pituitary under the renal capsule reversed this effect of hypophysectomy. Accordingly, the pituitary hormone involved, i.e. probably prolactin or MSH, should directly depress the activity of the 3β-hydroxysteroid dehydrogenase.

Longitudinal studies of serum proteins measured by radial immunodiffusion. Enzyme activity studies require almost always the sacrifice of the animal. Protein levels can be measured specifically in 5 μl serum by radial immunodiffusion [9]. Preference, therefore, was given to the latter type of endpoint. Rat transcortin and α_{2U}-globulin were purified, antibodies against these proteins were raised in rabbits and radial immunodiffusion assays were set up [10].

Prolactin levels were determined by E. Kühn (Leuven) using an homologous radioimmunoassay kit provided by NIAMDD. *Growth hormone* levels were measured through the courtesy of P. Franchimont (Liège).

RESULTS

1. *Transcortin levels in adulthood are affected by neonatal androgenisation* [11]

Three groups of male and female rats were castrated on the first day of life, treated on the second day and compared during adult life. The first group received an injection of 0.1 ml of olive oil on the second day of life, the second group got 1 mg of testosterone propionate under the same circumstances and the third group was injected with 200 μg of estradiol benzoate. On the 60th day of life both male and female rats in the first and in the third group had transcortin levels of about 150 mg/l., which is the normal adult female level. At the same time, animals in the group treated with testosterone propionate on the second day of life, had levels of about 100 mg/l., which is significantly lower than in the two other groups.

2. *Neonatal androgenisation seems to influence the response of transcortin to testosterone in adult female rats*

Rats of both sexes were gonadectomized on the first day of life and received on day 2 either 0.1 ml of olive oil subcutaneously or 1 mg of testosterone propionate (TP) in 0.1 ml of olive oil. From day 70 of life these animals received 7 daily injections of 50 μg TP per 100 g body weight subcutaneously. Transcortin levels were measured on day 77 (Table 2).

No effect was observed in males. After 7 days of TP treatment the animals treated neonatally with TP had the same transcortin levels as the animals treated neonatally with oil (74.1 $\pm$ 8.4 mg/l.; n:8 versus 75.9 $\pm$ 9.3 mg/l.: n:8). Under the same circumstances, however, a difference was observed in females: 72.2 $\pm$ 8.3 (S.D.) mg/l. in 8 rats treated with TP neonatally and 89.0 $\pm$ 7.4 (S.D.) mg/l. in 8 oil-treated controls ($P = 0.001$).

3. *Serum levels of transcortin in hypophysectomized rats implanted with pituitary tissue under the renal capsule*

Rats of both sexes were kept in standard conditions of temperature and humidity and received a constant pellet diet and tap water *ad libitum*. The animals were hypophysectomized on day 45 of life and received sucrose (10%) and NaCl (0.9%) in their drinking water

Table 2. Androgen responsiveness in adulthood of rats castrated neonatally and injected neonatally either with olive-oil (0.1 ml) or with testosterone propionate (1 mg in 0.1 mg olive oil)

Transcortin level (mg/l. ± S.D.)	neon. gx + oil		neon. gx + TP	
	adult oil	adult TP	adult oil	adult TP
Male				
mg/l. ± S.D.	122.9 ± 24.6	75.9 ± 9.3	87.5 ± 9.9	74.1 ± 8.4
	[4]	[8]	[8]	[8]
% of basal values	100	61.7	70.1	60.2
Female				
mg/l. ± S.D.	145.2 ± 41.4	89.0 ± 7.4	94.9 ± 8.5	72.2 ± 8.3
	[4]	[8]	[8]	[8]
% of basal values	100	61.4	65.4	50.0

for the rest of their life. Control animals were treated similarly. About day 80 of life hypophysectomized animals were implanted under the renal capsule either with a pituitary or with a piece of fat tissue (sham-implants). Thereafter, the animals were studied for protracted period during which serial blood samples were taken and body weights were recorded before and after various treatments. At autopsy organ weights were recorded as well. In the experiments to be reported here 10 animals were used: 5 male and 3 female hypophysectomized rats implanted with a pituitary, as well as 1 male and 1 female hypophysectomized sham-implanted animal.

Transcortin levels (*Figure* 1). Rats hypophysectomized on day 45 of life had transcortin levels of about 55 mg/l. on day 80 of life. The levels in male rats (55.3 ± 11.2 mg/l.) were not significantly different from the levels in female rats (56.1 ± 8.2 mg/l.). Pituitaries were implanted on day 80 and 3 weeks later transcortin levels in the 8 implanted rats had risen to 92 ± 7.7 (S.D.) mg/l. No differences were noted depending upon the sex of the donor or of the receptor animal: female rats implanted with a male pituitary reacted as male rats who had received a female pituitary graft, or as male or female rats who had received a pituitary from an animal of the same sex. The transcortin levels at the 106th day of life in these 4 groups of animals were respectively: 87.3, 85.8, 94.4, 101.7, 80.4, 101.7, 91.5, and 97.3 mg/l. In the two sham-operated rats the transcortin levels remained low and fluctuated between 44.4 and 62.3 mg/l. At the age of 140 days the 8 hypophysectomized-implanted animals as well as the sham-implanted rats received 200 μg of testosterone prepionate subcutaneously in olive oil for 7 consecutive days. Transcortin levels remained high (97.8 ± 13.9 mg/l.) in the implanted rats and low (48.1 and 52.4 mg/l.) in the sham-implanted rats. At the 204th day of life, the 8 implanted animals still had a mean transcortin level of 94.3 ± 6.0 mg/l. At that time the rats received subcutaneous injections of 100 μg of *estradiol benzoate* in olive oil for 7 consecutive days. On day 212

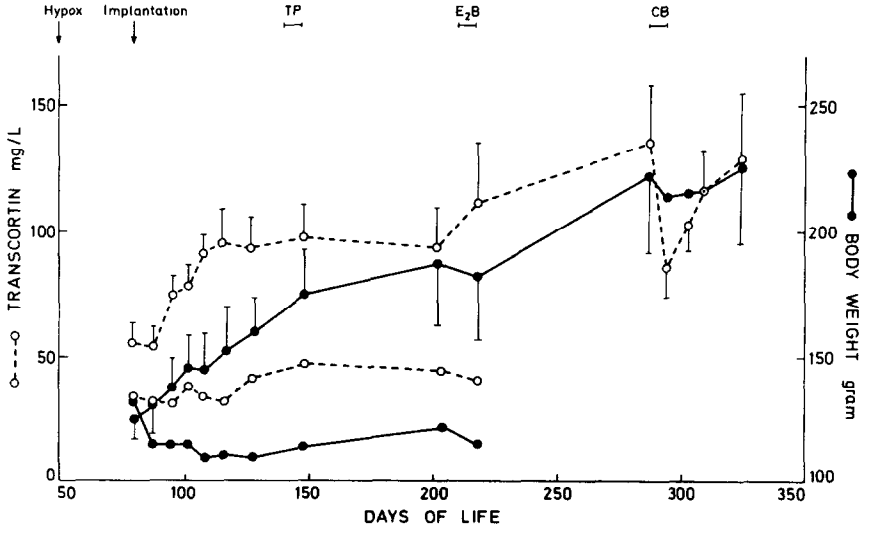

Fig. 1. Body weights (G ± S.D.) and transcortin levels (mg/l ± S.D.) of 8 hypophysectomized rats having a pituitary implanted under their renal capsule (2 upper lines) and of 1 female hypophysectomized rat with a piece of fat tissue implanted under the renal capsule (2 lower lines). The latter animal died about the 235th day of life. TP stands for testosterone propionate given subcutaneously for 7 days in a daily dose of 200 μg in olive oil, E_2B means estradiol benzoate given similarly for 7 days in a daily dose of 100 μg, and CB indicates that 250 μg of bromo-ergocryptine was given daily, suspended in olive oil, for 7 days.

of life transcortin levels had increased somewhat (112.8 ± 23.3 mg/l.) in the implanted rats but did not change in the control animals. In the implanted rats, the transcortin levels did not revert to the previous level but increased further and attained a mean value of 136.4 ± 22.1 mg/l. on day 287 of life. Next day bromo-ergocryptine (CB 154) was injected for 7 days, a daily dose of 250 μg being given subcutaneously suspended in olive oil. At the end of these 7 days the transcortin levels had decreased dramatically from 136.4 ± 22.2 mg/l. to 85.4 ± 10.9 mg/l. ($P < 0.01$). Thereafter, the transcortin levels increased gradually again over a period of three weeks, reaching a mean level of 102.3 ± 9.1 mg/l. eight days after the last injection and 129.4 ± 25.0 mg/l. two weeks later. Four of the 8 implanted rats then received a second series of CB injections. Again the transcortin levels fell significantly from 127.8 ± 8.6 to 85.8 ± 9.5 mg/l. ($P < 0.01$). The female control rat had died at that moment but transcortin levels in the male sham-implanted rat were hardly influenced by CB 154 treatment being 62.3 mg/l. before and 51.5 mg/l. after a first series of 7 daily injections and 57.1 mg/l. before and 58.4 mg/l. after a second series of 7 injections given a month later.

Body weights. In the male sham-implanted rat body weight increased from 130.6 g at day 80 of life to 166.6 g at day 340. In the female control rat body weight decreased from 132 g at day 80 to 110 g at day 220; this animal died about day 235 of life. In the 8 animals with pituitary transplants body weight increased continually to reach a plateau about the 290th day of life. The mean body weight was 125.0 ± 7.8 g at age 80 and 225.3 ± 30.5 g at the age of 324 days.

Hormone levels at autopsy. Serum prolactin levels were high (212.9 ± 83 ng/ml) in the 4 hypophysectomized implanted rats who did not receive CB 154 the 7 days before sacrifice, and were twenty times lower (10.5 ± 1.5 ng/ml) in the 4 similar animals treated with the latter drug. The growth hormone levels in the 8 implantated rats are given in Table 3. No clearcut pattern emerged. Noteworthly was the fact that the growth hormone levels in the hypophysectomized-implanted rats lay far above the detection limit of the radioimmunoassay used (1 ng/ml) but were not higher than the levels observed in hypophysectomized rats without pituitary implants (range: 0–37.3 ng/ml in 6 male rats and 7.2 to 31.8 ng/ml in 5 female rats).

Table 3. Growth hormone levels (ng/ml) and body weight (g in brackets) in 4 hypophysectomized-implanted rats killed at day 340 of life after treatment with bromo-ergocryptine (CB 154) and in 4 similar animals not treated with the latter drug before sacrifice at the same age

	CB 154	No CB 154
Males	2.2 (204.1)	11.9 (257.8)
	15.8 (269.8)	9.9 (218.1)
	18.5 (224.8)	
Females	11.0 (185.5)	36.5 (220.4)
		35.6 (280.7)

4. *Spontaneous increase of transcortin levels in some of the female hypophysectomized rats*

As a rule transcortin levels remain low in hypophysectomized animals. In some female animals, however, as already noted by Westphal [12], these low levels increase gradually. Out of 20 female hypophysectomized rats 4 showed such a spontaneous rise in transcortin levels, the mean level being 91.9 ± 6.0 mg/l. (instead of 56.1 mg/l.) at the age of 80 days. At that age one of these rats had a pituitary from a female donor rat implanted under the renal capsule. Before surgery the transcortin level in this animal was 95.7 mg/l. A week later this level had decreased dramatically to 42.8 mg/l. but thereafter increased slightly above the initial level reaching 109.3 mg/l. on day 106, 107.0 mg/l. on day 114, 106.7 mg/l. at day 119 and 107.5 mg/l. at day 127 of life. In contrast with the findings in the other hypophysectomized-implanted rats each of the subsequent treatments in this animal was followed *initially* by a clearcut decrease in transcortin levels. TP (200 μg/day, subcutaneously in olive oil) for 7 days induced a fall from 107.5 mg/l. to 90.1 mg/l. Seven daily injections of 100 μg estradiol benzoate (subcutaneously in olive oil) decreased the transcortin levels from 118.0 to 73.7 mg/l. but 3 weeks later a transcortin level of 136.5 mg/l. was noted. This level dropped to 93.8 mg/l. at the end of 7 daily injections of 250 μg of bromo-ergocryptine in olive oil. One month later at day 333 of life the transcortin level had again increased to 129.3 mg/l. After a second series of 7 daily injections of bromo-ergocryptine the rat was killed. At that time the transcortin level had fallen to 85 mg/l. On autopsy an hypophyseal remnant could clearly be seen in the sella tursica. The relative adrenal weight in this animal amounted to 0.009% whereas in other female hypophysectomized-implanted rats values of 0.004, 0.004 and 0.005% were found. Growth hormone in serum taken at autopsy was 5.2 ng/ml, prolactin levels were low (8.9 ng/ml) as expected after CB treatment. The body weight in this rat increased from 144 g at day 80 to 191 g at day 204 and 281 g at day 340 of life.

5. *Negative results for serum α_{2U}-globulin*

If our clustering was correct, α_{2U}-globulin should be regulated in another fashion as transcortin. This protein was studied in male hypophysectomized rats with or without a pituitary implanted under the renal capsule. Hypophysectomized rats, as already shown by Roy [4], did not have measurable α_{2U}-globulin levels. Implantation of a pituitary under the renal capsule did not induce the synthesis of this protein: the α_{2U}-globulin levels remained undetectable. This too was to be expected since implanted pituitaries do not produce growth hormone, ACTH or TSH and that the latter hormones must be present for andro-

genic induction of α_{2U}-globulin [13]. Testosterone treatment for 7 days (TP 200 μg daily) did not induce α_{2U}-globulin synthesis in a female hypophysectomized rat with a pituitary implanted under the renal capsule.

DISCUSSION

As far as transcortin levels are concerned our results suggest that in intact animals neonatal testosterone induces permanently, and adult testosterone temporarily, the secretion in the hypothalamus of an inhibiting factor. This factor depresses the secretion of prolactin(?) in the pituitary and so results in lower transcortin production in the liver(?). Uncoupling of the pituitary by transplanting the gland under the renal capsule results in an autonomous secretion of prolactin and accordingly high levels of transcortin that are independent of androgenic control.

Estrogens seem to stimulate directly, and permanently(?) the prolactin production in the pituitary since it increases transcortin levels in hypophysectomized-implanted rats but not in hypophysectomized rats. Bromo-ergocryptine (CB 154), an inhibitor of prolactin secretion in the pituitary [14, 15] decreased transcortin levels in the implanted animals but not in the hypophysectomized animal. It cannot be excluded that another pituitary hormone other than prolactin is involved since besides prolactin bromoergocryptine could influence the production of other hypophyseal hormones, e.g. MSH, that are under inhibitory control of the hypothalamus. Anyhow, growth hormone cannot account for the results obtained.

Female hypophysectomized rats sometimes show a spontaneous increase in transcortin. This seems to be due to the regeneration of an hypophyseal remnant. This remnant is still coupled to the hypothalamus as shown by the following facts. The relative adrenal weight in these rats is at least double that of hypophysectomized rats with persistent low transcortin levels. Furthermore, stress, as in intact rats, always induced, through corticosterone, a fall of transcortin levels in these animals. Male hypophysectomized animals showing a spontaneous rise in transcortin levels have never been described. This can be explained, if one admits that the regenerating hypophyseal remnants are always coupled to the hypothalamus and that the neonatally androgenized hypothalamus, even in the actual absence of androgen, produces enough inhibiting factor to suppress prolactin secretion in these pituitary remnants.

The hypophyseal factor affecting transcortin synthesis, and provisionally identified as prolactin, could also explain the effects of the pituitary on 3 other clusters, i.e. the clusters examplified by the microsomal 2α-hydroxylase of the liver [6] as well as the ones examplified by the cytosolic 5β-steroid reductase of the liver [7] and the cytosolic NADPH-dependent 3α-hydroxysteroid dehydrogenase of the kidney [8]. Prolactin should *depress* the synthesis of the two first clusters just mentioned and *stimulate* the synthesis of proteins in the third one. Basal levels of prolactin would be sufficient to influence (depress) the synthesis of the microsomal 2α-hydroxylase of the liver, whereas high estrogen-stimulated prolactin levels would be necessary to affect the last two clusters.

The synthesis of the α_{2U}-globulin is jointly regulated by gonadal steroids and the pituitary. However, if our clustering was correct, the synthesis of this globulin should be regulated in another fashion as transcortin. This proved to be so: hypophysectomized-implanted rat did not produce α_{2U}-globulin. Furthermore, α_{2U}-globulin synthesis is probably not permanently affected by neonatal androgen since this prolactin disappears from the serum of male adult rats after castration.

As a rule *body weight* remains low in hypophysectomized rats. Quite to our surprise, body weight increased 40% after implantation to attain 225 g at day 340 of life as compared to a body weight of 290 g in intact rats of the same age. The pituitary factor(s) responsible for (or permitting) this increase in body weight are probably not LH, FSH, ACTH or TSH since the latter hormones are secreted under the influence of releasing hormones of the hypothalamus. It is possible that the residual growth hormone levels found in the hypophysectomized implanted rats do explain the weight gain, but similar levels were found in hypophysectomized rats without implants which did not grow. In a recent article on catch-up growth in children following surgery for craniopharyngioma, Costin and collaborators [16] noted growth hormone deficiency but normal prolactin levels and somatomedin activity in the growing children.

REFERENCES

1. De Moor P., Verhoeven G. and Heyns W.: Permanent effects of foetal and neonatal testosterone secretion on steroid metabolism and binding. *Differentiation* **1** (1973) 241–250.
2. De Moor P., Adam-Heylen M., Van Baelen H. and Verhoeven G.: Effects of testosterone mediated or modulated by pituitary factors. *J. Endocr.* **67** (1975) 71–79.
3. Colby H. D., Gaskin J. H. and Kitay J. I.: Requirement of the pituitary gland for gonadal hormone effects on hepatic corticosteroid metabolism in rats and hamsters. *Endocrinology* **92** (1973) 769–774.
4. Roy A. K.: Androgen-dependent synthesis of α_{2U} globulin in the rat: role of the pituitary gland. *J. Endocr.* **56** (1973) 295–301.
5. Denef C.: Effect of hypophysectomy and pituitary implants at puberty on the sexual differentiation of testosterone metabolism in rat liver. *Endocrinology* **94** (1974) 1577–1582.
6. Gustafsson J. A. and Stenberg A.: Masculinisation of rat liver enzyme activities following hypophysectomy. *Endocrinology* **95** (1974) 891–896.
7. Ghraf R., Lax E. R. and Schriefers H.: The hypophysis in the regulation of androgen and oestrogen dependent enzyme activities of steroid hormone metabolism in rat liver cytosol. *Hoppe-Seyler's Z. Physiol. Chem.* **356** (1975) 127–134.

8. Ghraf R., Lax E. R., Hoff H. G. and Schriefers H.: The role of the gonads and the hypophysis in the regulation of hydroxysteroid dehydrogenase activities in rat kidney. *Hoppe-Seyler's Z. Physiol. Chem.* **356** (1975) 135–142.
9. Van Baelen H. and De Moor P.: Immunochemical quantitation of human transcortin. *J. clin. Endocrinol. Metab.* **39** (1974) 160–163.
10. Vandoren G. and Van Baelen H.: Quantitation of serum α_{2U}-globulin by radial immunodiffusion. Unpublished data.
11. Van Baelen H., Adam-Heylen M., Vandoren G. and De Moor P.: Neonatal imprinting of serum transcortin levels in the rat. Unpublished data.
12. Westphal U.: *Steroid Proteins interactions.* Springer-Verlag, Berlin, Heidelberg, New York. Chapter IX, p. 235–293.
13. Roy A. K. and Leonard S.: Androgen-dependent synthesis of α_{2U} globulin in diabetic rats: the role of insulin. *J. Endocr.* **57** (1973) 327–328.
14. Pasteels J. L., Danguy A., Frêcotte M. and Ectors F.: Inhibition de la secrétion de prolactine par l'ergocornine et la 2-Br-α-er-gocryptine: action directe sur l'hypophyse en culture. *Ann. d'Endocr.* (Paris) **32** (1971) 188–192.
15. Kuew-Hsiung Lu, Koch Y. and Meites J.: Direct inhibition by ergocornine of pituitary prolactin release. *Endocrinology* **89** (1971) 299–233.
16. Costin G., Kogut M. D., Phillips L. S. and Daughaday W. H.: Craniopharingioma: the role of insulin in promoting postoperative growth. *J. clin. Endocr. Metab.* **42** (1976) 370.

DISCUSSION

Friesen. I was very interested in your data on the effect of the implant on growth. Were you able to see this in both male and female or was it in just the one sex?

De Moor. There was no difference between the 5 males and the 3 females included in this group.

Friesen. A point which might bear on this finding is that we've been looking at the effects of prolactin on the induction of its own receptor in the liver, and have found that it can induce its own receptor more easily in the female; and what is even more interesting is that prolactin also stimulates the production of somatomedin by the liver.

De Moor. Some data of the literature may bear on this too. Costin *et al.* (*J. clin. Endocr. Metab.* **42**, 370, 1976), have shown that children operated upon for craniopharyngioma start growing again and this in the absence of radioimmunoassayable growth hormone. These authors ascribed this growth to hypothalamic hyperphagia and consequently hyperinsulinism. They noted, however, that prolactin and somatomedin levels were normal in the growing subjects.

Westphal. Did you see any effect of TSH or thyroid hormones in the culture system derived from hypophysectomized animals?

De Moor. We did not try thyroid hormone or TSH. The hepatoma culture (HTC) cells we used did not have an adenylcyclase system so it is difficult to imagine that they could respond to TSH. In fact we tried LH, FSH, and insulin in vain.

Posner. Have you had the opportunity to examine the level of transcortin in the rat in pregnancy and in rats with pituitary tumours, secreting large quantities of prolactin. And have you looked at the effect of estrogen in hypox versus intact rats.

De Moor. To answer your second question first: hypox rats do not respond to estrogen as far as transcortin is concerned. As to your second question: I did not have the opportunity to study the rats implanted with the pituitary tumours you are alluding to. Yes we studied transcortin levels during the last third of pregnancy both in mother and fetus (H. Van Baelen, G. Vandoren and P. De Moor, *J. Endocr.* (unpublished data). We observed a dramatic fall of serum transcortin both in mother and fetus and this from day 18 or 19 of pregnancy on. This decrease was even more pronounced when pregnant rats were treated with dexamethasone. Using total serum corticosterone concentrations published in the literature (Dupouy *et al.* *J. Endocr.* **65** (1975) 347–352 it can be calculated that the unbound corticosterone concentration increases from 7 nM on day 17 to 51 nM on day 21.

Skett. From your data it seems that the CB-154 acts on the implanted pituitary.

De Moor. Yes, that's our conclusion and this conclusion is based on the following facts. CB 154 does not lower transcortin levels below the 50–55 mg/l level in hypophysectomized rats, but it lowers transcortin from about 130 mg/l to about 85 mg/l in hypox-implanted rats.

Skett. The reason I ask this is that the work of Fuxe and co-workers seems to suggest that the CB-154 acts on the brain, rather than directly on the pituitary.

De Moor. I can tell you only that CB does not work on hypox animals but that it works in hypox-implanted animals.

Skett. One other point about the CB-154: we injected into rats and found that it had no effect on liver enzymes in intact female rats. So that even though the prolactin level was reduced substantially, to about 10% of the normal level, there was still no effect.

Naftolin. Dr. De Moor, the rat is one of the animals that has a very acute positive feedback to prolactin and this hypothalamic mechanism can be blocked by neonatal injection of androgen or estrogen. Your evidence makes one inclined to think that in regards to the "feminizing factors" the estrogen is working at the pituitary level, and that the blockage hypothalamic feedback has nothing to do with the effect of estrogen on the pituitary factor. You have shown that by hypophysectomy and giving the estrogen separately. I would like to ask Dr. Sonnenschein whether the pituitary tumour that was injected in Dr. Gustafsson's work is a prolactin secreting pituitary tumour generated by giving veterinarian quantities of estrogen to rats?

Skett. Perhaps I could answer Dr. Naftolin's question on the prolactin producing tumours of Dr. Sonnenschein. We tried 4 of Dr. Sonnenschein's tumours and there was only one of those four which produced prolactin (Skett P., Eneroth P., Gustafsson J.-Å. and Sonnenschein C.: Changes in hepatic steroid metabolism in rats following transplantation of four different clones of pituitary tumour cells. *Endocrinology* (1977) in press).