abnormal[17]. In addition, in the case of diabetes ketone bodies are accompanied by other metabolic disturbances which may act in concert to perturb the normal development of the fetus. The present results indicate that the fetal abnormalities associated with maternal diabetes may be linked not only to hyperglycemia but also to other metabolic disturbances. Thus the possibility is raised that other conditions such as hyperemesis gravidum, which can also cause ketosis, might disturb the normal development of the fetus. It would be interesting to determine whether a correlation exists between the occurrence of hyperemesis during human pregnancy and the incidence of congenital defects.

1 Acknowledgments. We wish to thank Mr C. Veltkamp and Mr B. Lewis for their expert assistance with the scanning electron microscopy and photography, Mr M. Smedley who suggested improvements to the manuscript, Miss A. Callaghan who prepared the manuscript, and an anonymous donor for financial support for E.A.S.

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0014-4754/85/020273-03$1.50 + 0.20/0

The effect of castration, testosterone and estradiol on ^{14}C-serotonin metabolism by organ cultures of male rat pineal glands

S. Daya and B. Potgieter

Department of Pharmacology and Therapeutics, Medical University of Southern Africa, P O Medunsa 0204 (Republic of South Africa), and School of Pharmaceutical Sciences, Rhodes University, Grahamstown (Republic of South Africa), 29 August 1983

Summary. The pineal gland of the male rat does not appear to rely on prior conversion of testosterone to estradiol for the stimulant effect of testosterone on pineal melatonin and 5-methoxytryptophol synthesis.
Key words. Rat pineal gland; pineal gland, rat; estradiol; testosterone; methoxyindoles; hydroxyindole-O-methyltransferase; castration; serotonin metabolism.

The gonadal sex steroids are known to have a feedback relationship with the pineal gland with regard to synthesis of 5-methoxyindoles[1]. Of these 5-methoxyindoles, melatonin and 5-methoxytryptophol have been shown to possess antigonadotrophic activity. That such feedback exists has been demonstrated in previous studies which show that castration results in a significant decrease in activity of the enzyme responsible for synthesis of 5-methoxyindoles in the pineal gland namely, hydroxyindole-o-methyltransferase (HIOMT), in both male[2] and female[3] rats. Administration of both low doses of testosterone to castrated male[2] rats and estradiol to castrated female[3] rats results in restoration of HIOMT activity to that of control values and higher. Since these 5-methoxyindoles possess antigonadotrophic[4,5] activity, they play an important role in the reproductive cycle. It is therefore also of importance to know how the sex steroids modulate the synthesis of the 5-methoxyindoles by the pineal gland. The pineal gland of the male rat is readily able to convert testosterone to estradiol[6]. However it is not known as to what extent the pineal gland of the male rat relies on this conversion for modulation of 5-methoxyindole synthesis. The modulatory effect of testosterone could therefore be an indirect one. We therefore decided to investigate the extent to which the pineal gland of the male rat depends on the conversion of testosterone to estradiol with regard to the synthesis of 5-methoxyindoles.

Materials and methods. 5-Hydroxy (side-chain-2-^{14}C) tryptamine creatinine sulphate was purchased from Amersham, England; Testosterone propionate, estradiol and the 5-methoxyindoles from Sigma, 0.25 mm GF254 TLC plates from Merck, Germany. BGJb culture medium from Gibco Europe. Male rats of the Wistar strain (230–250 g) were castrated or sham-operated 2 weeks prior to use and were housed under a light cycle of LD 12:12 with food and water ad libitum. The rats were killed by neck fracture at 09.00 h and the pineal

The effect of castration, 10 nM testosterone and 10 nM estradiol on conversion of ^{14}C-serotonin to ^{14}C-5-methoxyindoles by organ cultures of male rat pineal glands (dpm/20 µl medium/gland ± SEM)

5-Methoxyindole	Sham-operated	Castrated	10 nM testosterone	10 nM estradiol
Melatonin	441.2 ± 18[a]	286.7 ± 8[a,c]	454.2 ± 12[c]	205.7 ± 25
5-Methoxytryptophol	1069.7 ± 39[b]	721.7 ± 12[b,d]	981.4 ± 35[d]	840.1 ± 46
5-Methoxyindoleacetic acid	434.0 ± 37	484.6 ± 26[e,f]	545.0 ± 13[e]	653.4 ± 4[f]

[a] $p < 0.005$; [b] $p < 0.001$; [c] $p < 0.005$; [d] $p < 0.02$; [e] $p < 0.05$; [f] $p < 0.001$ (Student's t-test).

glands were removed aseptically and placed into 1-cm diameter sterile petri dishes containing 200 µl of BGJb culture medium (Fitton-Jackson modification). Each petri dish contained 5 pineal glands each. To each petri dish, 2 µCi of ^{14}C-5-hydroxytryptamine creatinine sulphate was added[7], followed by either vehicle or steroid. The petri dishes were then immediately placed in an incubator at 37 °C with a humidity of 95% and an atmosphere of 5% CO_2:95% O_2 for a period of 24 h. After the 24-h culture period, the pineal glands were removed from the petri dishes and 20 µl of the culture medium was spotted in duplicate together with 4 µg each of synthetic unlabeled 5-methoxyindoles. The plates were then developed twice in the same direction using chloroform, methanol and acetic acid (93:7:1) as the first solvent and once in the second direction using ethyl acetate[8]. The plates were dried with a stream of nitrogen, the spots were visualized under UV light and scraped into scintillation vials and the radioactivity measured. Differences between duplicates was not greater than 12%.

Results. Castration decreases the ability of the pineal glands to synthesize melatonin and 5-methoxytryptophol (5-MTOH). The addition of 10 nM testosterone to the culture medium restores the ability of the pineal glands from the castrated male rats to synthesize the 5-methoxyindoles to values similar to those of the sham-operated controls.

The addition of 10 nM estradiol to the culture medium results in increased synthesis of 5-methoxyindoleacetic acid (5-MIAA) but does not alter synthesis of 5-MTOH or melatonin.

Discussion. These results indicate that castration results in a decrease in the ability of the pineal glands to synthesize 5-methoxyindoles. The addition of testosterone results in restoration of the decrease to control values, implying that testosterone is possibly involved in a feedback relationship with the pineal gland. These results are in keeping with previous studies which have shown that administration of low doses of testosterone to castrated male rats restores the decrease in pineal HIOMT activity to that of control values[2]. Since estradiol, unlike testosterone, was unable to elevate the levels of melatonin and 5-MTOH, it could be that estradiol does not stimulate HIOMT for the synthesis of those methoxyindoles in the male rat pineal gland. Another explanation could be the lack of effect of estradiol on the rate-limiting enzyme involved in the synthesis of melatonin in the pineal, namely N-acetyl transferase[9] while testosterone on the other hand is able to stimulate the depressed activity of this enzyme in castrated male rats[10]. It appears that the pineal gland of the male rat does not rely on prior conversion of testosterone to estradiol for the stimulant effect of a physiological concentration of testosterone on pineal melatonin and 5-MTOH synthesis. It appears therefore that testosterone has a direct effect on these pathways.

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0014-4754/85/020275-02$1.50 + 0.20/0

Hormonal induction of steroid sulphatase in the mouse

S.T.S. Lam* and P.E. Polani

Paediatric Research Unit, Prince Philip Research Laboratories, Guy's Hospital Medical School, Guy's Tower, London Bridge SE19RT (England), 25 July 1983

Summary. By comparing steroid sulphatase levels per se, and also ratios to α-galactosidase, in 6 sets of mice – normal females, entire and castrated males both with and without exogenous testosterone administration – we obtained support for the contention that induction of this enzyme is in part controlled by male hormones.
Key words. Steroid sulphatase; male hormones; mouse.

We have shown that in the mouse[1,7], as opposed to Man[11], the wood lemming[17], and the horse[13], steroid sulphatase (STS) activity, as measured in two different tissues at two different ages[7] is not increased in females compared with males. Assuming that the activity of this enzyme reflects closely the number of (structural) gene loci directly responsible for its production and effect, such a result suggests one of the following alternatives: either the gene locus resides on the X chromosome – as is the case for other mammals – and takes part in the random inactivation of this chromosome, so that females and males virtually have the same dose of steroid sulphatase gene *(STS)*, namely one; or else the locus is autosomal, and males and females both have two doses of the relevant gene. We should note, however, that evidence favors X-linkage of *STS* also in the mouse[4], in keeping with the general 'rule' that X-chromosomal genes are conserved in mammals.

As mentioned above, in Man, females have higher levels of STS activity, and it is on this dosage ground that *STS* has been deemed to escape random inactivation[12,14]. The double dose of the *STS* gene in females, compared to the single dose in males, should result in a double enzyme activity in the former. However, the female to male STS activity ratio falls consistently short of the expected 2:1 value, and is around 1.8:1. We have suggested that male hormonal influences may be a factor in decreasing the said difference between sexes, but partial inactivation of the gene in females could play a role also[10]; such an effect is not implausible if *STS* is located close to the watershed between differential and pairing segments of the X, as we think must be the case[12]. In our experiments on mice, we have shown a tendency to higher steroid sulphatase activity in male mice, at least in one age group. This would be in keeping with a male hormonal influence on gene expression, so that STS would be partly 'testosterone'-inducible.

We attempt here to assess the influence of testosterone on *STS* expression in the mouse, both in its 'physiological' stabilized state, and under exogenous stimulation. The effect of exogenous testosterone on induction of β-glucuronidase and α_1-antitrypsin activities in different strains of mice have been docu-