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.

1 Preslock, J.P., Life Sci. *20* (1977) 1299.
2 Nagle, C.A., Cardinali, D.P., and Rosner, J.M., Neuroendocrinology *14* (1974) 14.
3 Cardinali, D.P., Nagle, C.A., and Rosner, J.M., Hormone Res. *5* (1974) 304.
4 Collu, R., Fraschini, F., Vasoconti, P., and Martini, L., Endocrinology *90* (1972) 1231.
5 McIsaac, W.M., and Page, I., J. biol. Chem. *234* (1959) 858.
6 Cardinali, D.P., Nagle, C.A., and Rosner, J.M., Experientia *30* (1974) 1022.
7 Cardinali, D.P., Vacas, J.M., and Ritta, M., Experientia *37* (1981) 203.
8 Klein, D.C., and Notides, A., Analyt. Biochem. *31* (1969) 480.
9 Illnerova, H., Endocr. exp. *9* (1975) 141.
10 Daya, S., and Potgieter, B., S.A.J. Science *78* (1982) 174.

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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-

mented[6,16]. The mechanism of protein induction is mostly due to a modification of the transcriptional process, and other controls at the cellular level are also important. This is exemplified by the failure of β-glucuronidase induction in mice with testicular feminization (tfm;[15]), in which the cytosol receptor for testosterone is defective.

Dehydroepiandrosterone sulphate (DHEAS) is used as the substrate, and α-galactosidase (α-gal; X-linked and subject to inactivation in the female mouse[8]: see Discussion) as a marker enzyme. Arylsulphatase C (araC) activity, using the artificial substrate β-methylumbelliferyl sulphate, correlates closely to that of STS assayed against DHEAS. Indeed, identity of the two enzymes has been suggested. Our experience is borne out by other work[9], but it is unclear whether one is assaying the same enzyme when two different substrates are employed[2,3].

Materials and methods. Thirty adult mice, aged between 17½ and 28 weeks, of the C3H strain, were used, divided into 6 groups (table 1), each group comprising age-matched animals. Groups C1 and C2 were castrated under ether anesthesia, 12 weeks prior to treatment. All males had been kept away from females for at least one month prior to injection, and each group was housed in a separate cage. Intraperitoneal injections, using a 23 gauge needle, were given to all the mice at the same time. On days 1, 3, 5, 7 and 9, the ethyl oleate group (controls for the testosterone groups) received doses of 0.25 ml of ethyl oleate. The testosterone groups received 0.25 mg of testosterone propionate in 0.25 ml ethyl oleate. All the mice were killed by cervical dislocation on day 12, and the livers dissected out and homogenized in 0.32 M Sucrose (1:10 for w/v), in a 10 ml Elvehjem homogenizer. Enzymes were assayed on the whole homogenate: STS and α-gal as described by Lam et al.[7], and araC by a modification of the method of Hameister et al.[5]. 20 µl of the sample was incubated with 100 ml of 1 mM 4-methylumbelliferyl sulphate in 0.1 M Tris buffer at pH 7.5 for one hour; the reaction was then stopped by cooling to 0 °C and adding 1.5 ml of 0.5 M glycine-NaOH, pH 10.5, and the umbelliferone liberated was then read from a fluorescence spectrometer. All samples were tested in duplicate.

Results. Entire males, females and castrated male mice that received testosterone have a slightly higher mean enzyme activity (table 2). For α-gal, there is no statistical significance in this difference in any of the three groups. This is also true for STS and araC activities in the female and castrated male groups. However, in the male mice, there is a statistically higher activity of both STS and araC among those treated with testosterone. When steroid sulphatase is expressed in relation to α-gal, the different groups show the distributions plotted in figure 1. The testosterone-treated sets in each of the three groups of mice show a higher mean value compared with untreated controls, but the differences are not significant. The mean values also tend to be higher in the normal male mice than in the other two groups of mice. Although the difference between groups F1 and M1 is not significant, there is a significant difference between groups F1 and M2 ($0.02 > p: > 0.01$ by Student's t-test), and between F2 and M2 ($0.05 > p > 0.025$). It is noteworthy that the two groups of castrated males, especially C2, show wide variation of values around the mean, and there is no significant difference between C1, C2 and any other group.

When araC activity is expressed in relation to α-gal for each sample, the picture is similar (fig. 2). Here the testosterone-treated groups in each of the three types of mice again show no

Table 1. Grouping of experimental mice

Group and sex		Number	Treatment
F1	Normal female	5	Ethyl oleate
F2	Normal female	5	Testosterone in ethyl oleate
M1	Normal male	6	Ethyl oleate
M2	Normal male	5	Testosterone in ethyl oleate
C1	Castrated male	4	Ethyl oleate
C2	Castrated male	5	Testosterone in ethyl oleate

Table 2. Enzyme activities in the six groups of mice

	Steroid sulphatase activity (pmole/mg protein/h)	Arylsulphatase activity (nmole/mg protein/h)	α-Galactosidase activity (nmole/mg protein/h)
Group F1 (n = 5)	136.1 ± 37.5	8.65 ± 1.02	23.44 ± 2.2
Group F2 (n = 5)	157.9 ± 20.6	9.7 ± 0.8	24.6 ± 1.8
Difference between F1 and F2	$p > 0.2$	$p > 0.1$	$p > 0.2$
Group M1 (n = 6)	128.76 ± 19.1	8.77 ± 0.26	17.13 ± 1.3
Group M2 (n = 5)	174.8 ± 23.3	10.34 ± 1.4	20.95 ± 4.98
Difference between M1 and M2	$p < 0.01$	$p < 0.05$	$p > 0.1$
Group C1 (n = 4)	145.84 ± 19.7	8.99 ± 0.8	23.9 ± 2.7
Group C2 (n = 5)	167.5 ± 43.0	10.1 ± 1.5	24.4 ± 3.5
Difference between C1 and C2	$p > 0.5$	$p > 0.2$	$p > 0.5$

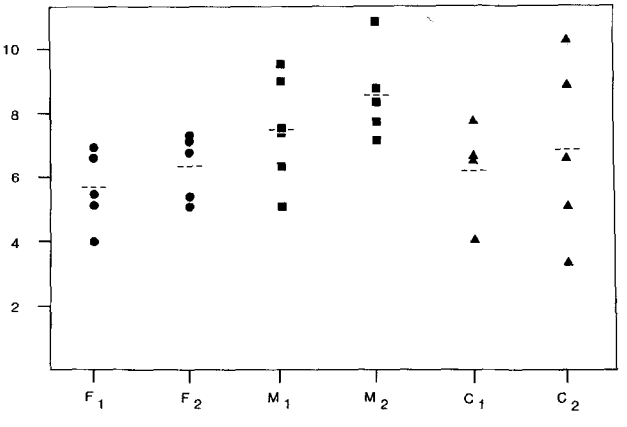

Figure 2. Distribution of ratios of arylsulphatase C to α-galactosidase in the different experimental groups (see table 1 and text). × 1000.

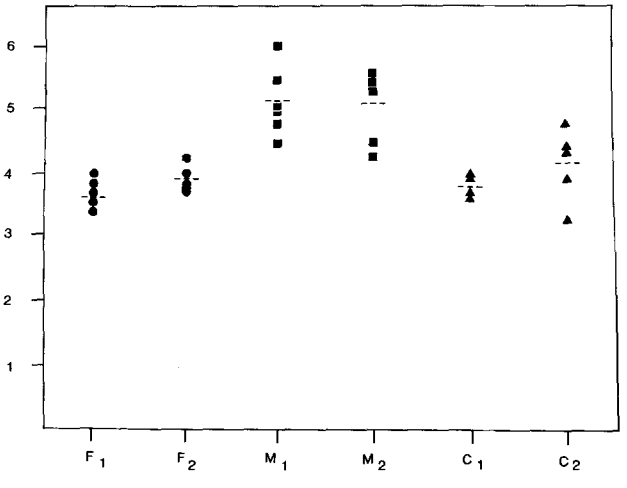

Figure 1. Distribution of ratios of steroid sulphatase to α-galactosidase in the different experimental groups (see table 1 and text). × 1000.

difference compared with their controls, but both normal male groups show significantly higher values than the rest (e.g. M1 vs C1: $0.005 > p > 0.001$; M1 vs C2: $0.05 > p > 0.025$). The female and castrated male groups have similar values.

Discussion. The overall picture is interpretable as pointing to a male hormonal influence as playing a role in the induction of STS activity in the mouse liver. This conclusion can be reached despite the methodological limitation that enzyme activities cannot be assessed before and after hormone treatment in the same animal, in view of the tissue assayed. Certainly the development of more sensitive enzyme assays, for example, microtechniques that would allow tests to be done on small amounts of tissues such as whole blood, would be of help. In addition, it is probable that the dose of testosterone used was not the most effective, considering also the long interval between castration and the execution of the experiments reported here.

In our experiments, the effect of endogenous male hormones on enzyme induction is not apparent, when considering the absolute levels of STS in castrated and normal males: in fact the castrated group have higher – though not significantly so – mean values than normal controls. However, the situation is reversed when the enzyme activity is expressed in relation to the marker enzyme α-gal. Such a way of looking at STS activity (and that of araC) is especially appropriate as the *α-gal* gene is subject to inactivation when one of the female X chromosomes is randomly inactivated. Here, females and castrated males have similar ratios, and these are lower than in entire males. In each set, the ratios rise in those treated with testosterone injections, compared with the control animals. The effect of exogenous testosterone on induction of the enzyme is evident. It is to be noted that the effect on the castrated mice shows wide variation in response, and this could be due to an altered metabolic state after castration. These results are barely evident statistically in the present study, and this is compounded of small sample size and individual variation of enzyme levels, as well as, possibly, other factors discussed above. Finally, a relevant question concerns the role of testosterone in enzyme induction. By analogy to β-glucuronidase induction in the murine kidney, it is not implausible to suggest a form of regulatory action at the level of transcription. Confirmatory evidence for this would require studies of mRNA specific for the protein concerned, and information of this nature will be of importance in our understanding of the regulation of gene expression at this locus.

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* Current address: Paediatrics 'A' Unit, Queen Elizabeth Hospital, Kowloon, Hong Kong.

1 Crocker, M., and Craig, I., Variation in regulation of steroid sulphatase locus in mammals. Nature *303* (1983) 721.
2 Dolly, J.O., Dodgson, K.S., and Rose, F.A., Studies on the oestrogen sulphatase and arylsulphatase C activities of rat liver. Biochem. J. *128* (1972) 337.
3 French, A.P., and Warren, J.C., Properties of steroid sulphatases and arylsulphatase activities of human placenta. Biochem. J. *105* (1967) 233.
4 Gartler, S.M., and Rivest, M., Evidence for X-linkage of steroid sulfatase in the mouse: steroid sulfatase levels in oocytes of XX and XO mice. Genetics *103* (1983) 137.
5 Hameister, H., Wolff, G., Lauritzen, C.H., Lehmann, W.O., Hauser, A., and Ropers, H.H., Clinical and biochemical investigations on patients with partial deficiency of placental steroid sulfatase. Hum. Genet. *46* (1979) 199.
6 Kueppers, F., and Mills, J., Trypsin inhibition by mouse serum: sexual dimorphism controlled by testosterone. Science *219* (1983) 182.
7 Lam, S.T.S., Polani, P.E., and Fensom, A.H., Steroid sulphatase in the mouse. Genet. Res. (Camb.) *41* (1983) 299.
8 Lusis, A.J., and West, J.D., X-linked inheritance of a structural gene for α-galactosidase in *Mus musculus*. Biochem. Genet. *14* (1976) 849.
9 Meyer, J.C., Weiss, H., Grundmann, H.P., Würsch, T.G., and Schnyder, U.W., Deficiency of arylsulfatase C in cultured skin fibroblasts of X-linked ichthyosis. Hum. Genet. *53* (1979) 115.
10 Migeon, B.R., Shapiro, L.J., Norum, R.A., Mohandas, T., Axelman, J., and Dabora, R.L., Differential expression of steroid sulphatase locus on active and inactive human X chromosome. Nature *299* (1982) 838.
11 Müller, C.R., Migl, B., Traupe, H., and Ropers, H.H., X-linked steroid sulfatase: evidence for different gene-dosage in males and females. Hum. Genet. *54* (1980) 197.
12 Polani, P.E., Pairing of X and Y chromosomes, non-inactivation of X-linked genes, and the maleness factor. Hum. Genet. *60* (1982) 207.
13 Polani, P.E., Lam, S.T.S., Allen, W.E., and Pidduck, H., Steroid sulphatase in the horse. Manuscript in preparation (1984).
14 Ropers, H.H., Migl, B., Zimmer, J., Fraccaro, M., Maraschio, P., and Westerveld, A., Activity of steroid sulphatase in fibroblasts with numerical and structural X chromosome aberrations. Hum. Genet. *57* (1981) 354.
15 Shire, J.G.M., The forms, uses and significance of genetic variation in endocrine systems. Biol. Rev. *51* (1976) 105.
16 Swank, R.T., Paigen, K., and Ganschow, R.E., Genetic control of glucuronidase induction in mice. J. molec. Biol. *81* (1973) 225.
17 Wiberg, U., Mayerova, A., Müller, U., Fredga, K., and Wolf, U., X-linked genes of the H-Y antigen system in the wood lemming *(Myopus schisticolor)*. Hum. Genet. *60* (1982) 163.

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Methylation of hippocampal phosphatidylethanolamine and proteins during long-lasting potentiation

A.M. Benjamin, J.W. Goh and B.R. Sastry[1]

Neuroscience Research Laboratory, Department of Pharmacology and Therapeutics, Faculty of Medicine, The University of British Columbia, 2176 Health Sciences Mall, Vancouver (British Columbia V6T1W5, Canada), 10 October 1983

Summary. Whereas the monomethylation of hippocampal phosphatidylethanolamine is decreased following the induction of long-lasting potentiation of the CA_1 population spike, carboxymethylation of proteins is unaffected.

Key words. Rat hippocampus; hippocampus, rat; potentiation, long-lasting; carboxymethylation, protein; methylation, phospholipid; phosphatidylethanolamine.