Influence of cyproterone acetate on the activity of β-glucuronidase in the hypothalamus and cerebral cortex of the male mouse

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Summary. Daily s.c. injections of cyproterone acetate greatly decrease the protein content and β-glucuronidase activity in the mouse hypothalamus. These effects are reversible and the recovery capacity of the animal seems to be inversely related to the duration of antiandrogenic treatment.

It is widely recognized that the hypothalamus plays an important role in the control of reproductive activity. It is also a central target organ for sex hormones. The latter, in fact, are selectively incorporated in the hypothalamus and undertake a prominent part in the regulation of hypothalamic metabolic activity as well[2]. Little is known, however, of the steroid dependency of several biochemical parameters, one of which might be represented by the hydrolytic enzyme, β-glucuronidase (EC 3.2.1.31)[3-6]. Here we report the effects of an antiandrogen, cyproterone acetate (CA), on the activity of β-glucuronidase in the hypothalamus and cerebral cortex of the male mouse (Swiss albino cc).

Sexually-experienced male mice, at 45 days of age, received daily s.c. injections of CA (0.1 mg/day) (a gift from Schering, Berlin). All experimental animals were reared in groups of 5 mice each per cage. They had free access to food and water. Controls were reared under similar laboratory conditions. Samples of 5 animals each were sacrificed at 15, 30, 60, 90, 120 and 180 days after CA treatment, as well as parallel controls. Groups of 5 mice each, treated with CA for 30 and 90 days, were also subjected for a month to a daily 5α-dihydrotestosterone (DHT) therapy (50 μg/day). In addition to this in groups treated for 30, 90 and 120 days, the CA administration was suspended for 30 and/or 90 days. Each animal was weighed and killed with an overdose of ether. The hypothalamus and cerebral cortex were quickly dissected out, weighed and homogenized for enzyme assay. β-Glucuronidase activity was assayed as described earlier and expressed as nmole phenolphthalein liberated/μg protein/min[4]. Proteins were determined according to Lowry et al.[7]. Results were analyzed for significance using Student's t-test.

β-Glucuronidase activity already decreased significantly after 15 days of antiandrogenic treatment (table). A progressive decline of the enzyme activity was found to occur 30 days and then 180 days after antiandrogenic treatment. The protein content, after the initial lag of 15 days, showed a significant decrease due to cyproterone acetate. Daily injections of DHT for 30 days in mice treated with CA for 30 days stimulated both protein content and enzyme activity. In contrast, when the androgen therapy was given to animals treated for 90 days with CA both parameters seemed to remain largely unaffected. Suspension of antiandrogenic administration after a term of 30 days induced a progressive recovery of both protein content and β-glucuronidase activity. When CA administration was suspended after 90 or 120 days, for 30 or 90 days the antiandrogenic influence persisted, which may indicate that longer terms are probably required for obtaining a recovery of the 2 parameters examined here. It is known that, after reaching sexual maturity, the active reproductive life of male mice does not exceed 6-7 months. As a consequence we considered it logical not to study the enzyme activity following DHT-therapy or CA suspension in animals treated with CA

Effects of long-term treatment with cyproterone acetate on mouse hypothalamus and cerebral cortex

Experimental groups	Hypothalamus		Cerebral cortex	
	Protein (μg/mg tissue)	β-Glucuronidase	Protein (μg/mg tissue)	β-Glucuronidase
Initial control	86.8 ± 5.42	6.42 ± 0.78	132 ± 14	0.66 ± 0.08
CA 15 days	106.8 ± 7.23	2.16 ± 0.34*	128 ± 18	0.76 ± 0.09
Control (age 60 days)	89.2 ± 4.21	6.55 ± 0.60	134 ± 12	0.63 ± 0.09
CA 30 days	45.6 ± 3.46*	1.00 ± 0.09*	117 ± 22	0.70 ± 0.06
Control (age 75 days)	86.1 ± 4.74	6.34 ± 0.42	120 ± 16	0.66 ± 0.04
CA 60 days	58.4 ± 4.21*	1.30 ± 0.11*	124 ± 18	0.68 ± 0.07
Control (age 105 days)	87.0 ± 3.28	6.49 ± 0.80	122 ± 14	0.61 ± 0.06
CA 90 days	48.4 ± 2.18*	1.18 ± 0.10*	141 ± 26	0.65 ± 0.07
Control (age 135 days)	86.4 ± 4.05	6.18 ± 0.72	129 ± 17	0.64 ± 0.05
CA 120 days	31.2 ± 2.71*	1.01 ± 0.07*	121 ± 16	0.72 ± 0.07
Control (age 165 days)	85.8 ± 5.24	5.22 ± 0.56	128 ± 21	0.67 ± 0.05
CA 180 days	27.4 ± 1.92*	0.76 ± 0.06*	132 ± 20	0.68 ± 0.04
Control (age 225 days)	87.3 ± 4.51	3.61 ± 0.63	118 ± 20	0.67 ± 0.06
CA 30 days:				
+ DHT 30 days	64.8 ± 5.24**	2.92 ± 0.18**	122 ± 23	0.71 ± 0.05
+ 30-day recovery	55.0 ± 3.26**	1.54 ± 0.13**	127 ± 22	0.69 ± 0.05
+ 90-day recovery	79.5 ± 6.59**	2.36 ± 0.20**	134 ± 26	0.73 ± 0.08
CA 90 days:				
+ DHT 30 days	48.4 ± 4.42	1.31 ± 0.12	128 ± 18	0.68 ± 0.08
+ 30-day recovery	32.5 ± 2.26**	0.90 ± 0.07**	133 ± 24	0.74 ± 0.06
+ 90-day recovery	33.2 ± 2.34**	1.22 ± 0.09	120 ± 22	0.66 ± 0.08
CA 120 days:				
+ DHT 30 days***	30.4	0.98	125	0.68
+ 30-day recovery	30.3 ± 1.84	0.81 ± 0.04**	130 ± 24	0.69 ± 0.07

Each value is the mean ± SEM.
* Significance of difference vs control ($p < 0.01$).
** Significance of difference vs CA-treated ($0.02 < p < 0.01$).
*** Determination made on 1 animal.

for 180 days. The table shows, in fact, that in controls, at the age of 225 days there is a significant reduction of β-glucuronidase activity in the hypothalamus ($p < 0.02$).
In the cerebral cortex none of these treatments had any effect either on protein concentration or on β-glucuronidase activity, which apparently convalidates the specificity of the effects observed in the hypothalamus.
These results may mean that β-glucuronidase activity in the hypothalamus of male mouse is androgen-dependent. Firstly because the antiandrogen greatly decreases its activity and secondly the androgen-replacement therapy stimulates it. The androgen-dependence of β-glucuronidase activity in the hypothalamus is not restricted to mammalian species, however. We earlier demonstrated this phenomenon in the hypothalamus of the male frog[4]. Furthermore the effects of CA seem to be reversible, although longer CA treatments need longer recovery periods. These data also indicate that the recovery capacity of the animal after withdrawal of CA seems to be proportional to the duration of recovery, and inversely related to the duration of treatment.

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A human calcitonin-like molecule in the ultimobranchial body of the amphibia *(Rana pipiens)*

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Summary. As assessed by radioimmunoassay, and high performance liquid chromatography (HPLC), frog ultimobranchial calcitonin was found to be similar to synthetic human calcitonin but completely different from synthetic salmon calcitonin.

Frogs have a well developed ultimobranchial gland (UBG)[2] which is thought to contain calcitonin (CT). Extracts of frog UBG may induce hypocalcemia in a rat bioassay for CT[3]. Induced hypercalcemia will increase the secretory activity[4], while ultimobranchialectomy leads to short-lived hypercalcemia, hypercalciuria, increased osteoclastic bone activity and eventual osteopenia[5]. There is a marked seasonal variation in the activity of the UBG with maximal activity in the summer months while much less in the winter[6,7].
Amphibian growth hormone and prolactin are immunochemically related to rat growth hormone[8]. And toad LHRH is identical by radioimmunoassay and chromatography criteria to mammalian LHRH yet different from more nearly related species[9]. We have studied the immunochemical characteristics of frog UBG calcitonin to clarify whether it is more closely related to the primate-rodent group or to the teleost group of calcitonins.
Materials and methods. Adult frogs *Rana pipiens*, of both sexes were obtained in November, before the hibernation, from T. Gerrard & Co., Surrey, England. Groups of 25 were anesthetized with ether, and the UBG in the adjacent area to the glottis was removed, immediately frozen and stored at $-20\,^{\circ}C$. Pooled UBG were extracted and prepared for RIA as described before[3], except that the tissue was extracted first by 0.1 M HCl, 1% NaCl.
Human CT RIA was performed using 2 antihuman CT antisera. Antiserum 827/4 has mainly a mid molecule specificity, while antiserum 336/6 has both C-terminal and mid portion specificities[11]. Standards and labelled hormone of synthetic human CT and assay procedure were as described before[11]. Antiserum 827/4 was used in a dilution of 1:60,000, gave a sensitivity of 8 pg/tube after 5 days of incubation and antiserum 336/6 was used in a dilution of 1:24,000[13]. Salmon CT RIA was done as previously described[14]. Specific antibody to synthetic salmon CT was a gift from Dr L.J. Deftos, used in a final dilution 1:25,000. In addition the extract was applied to a reverse phase high performance liquid chromatography (HPLC)[15] and the fractions were assayed for both human CT and salmon CT immunoreactivity.
Results and discussion. Approximately 80% of labelled CT can be recovered by this hydrophilic tissue extraction method. Frog UBG's contained significant quantities of human CT-like immunoreactivity. The mean level was 16 ng/UB region, expressed as human CT. Extracts gave an

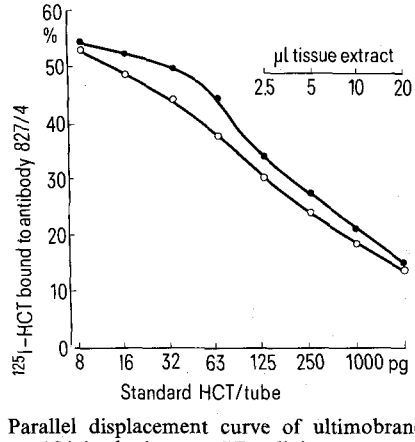

Figure 1. Parallel displacement curve of ultimobranchial gland tussue extract (●) in the human CT radioimmunoassay. Synthetic human calcitonin (○). Antiserum 827/4.