TESTOSTERONE METABOLISM BY SERIALLY SUBCULTURED FIBROBLASTS FROM GENITAL AND NONGENITAL SKIN OF INDIVIDUAL HUMAN DONORS

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SUMMARY

Skin fibroblasts were cultured from the prepuce and deltoid area of three males, and from the labia majora and abdomen of two females. The genital skin strains metabolised testosterone much faster than those derived from nongenital skin. This is the first clear demonstration of the persistence in serially subcultured cells of a metabolic difference between nonmalignant somatic tissues of human beings.

INTRODUCTION

Fresh skin slices from the prepuce, scrotum, clitoris and labium majus (the perineum) metabolise testosterone at a greater rate than slices obtained from the mons and various other areas of nongenital skin(1). These results have been confirmed by others for skin from the scrotum (2), labium majus (2), and prepuce (3) of adults, neonatal prepuce (4), and the "perineum" of male and female fetuses (5).

Testicular feminization (TF) is a gene-determined form of male pseudohermaphroditism in man. Neither the refractoriness to testosterone

of these patients (6), nor its mode of inheritance are understood (7). The ability to detect an expression of the mutant gene responsible for TF in cultured skin fibroblasts would be useful for investigating the molecular basis of testosterone refractoriness, and for distinguishing, as a consequence of X chromosome inactivation (8), between the sex-linked recessive and sex-limited autosomal dominant possibles modes of inheritance.

With these ultimate objectives in mind, we undertook a study of the metabolism of testosterone by serially subcultured monolayer fibroblast strains derived from genital (foreskin, labium majus) and nongenital (deltoid area of the arm, abdomen) skin of normal males and females at various ages. This report deals only with the rate of testosterone metabolism by such strains. The sequential pattern of testosterone metabolism observed and differences of the pattern in relation to the age and sex of the donor will be described separately (9).

MATERIALS AND METHODS

Pieces of labium majus and abdominal skin were obtained during gynecologic surgery, preputial skin at the time of circumcision, and deltoid skin by 4 mm punch biopsy. The strains were established and maintained according to Krooth (10). Plastic petri dishes (Falcon), 60 mm in diameter, were innoculated with 0.75 - 1.0 x 10^6 cells, a near-confluent population. Three or four days later, when mitosis had essentially ceased, replicate monolayers were washed with Earle's BSS and fed 2 ml. of Eagle's MEM supplemented with pyruvate and non-essential amino acids, each at 1 mM, and 2 x 10^5 dpm of testosterone - 4 -^{14}C (59.2 mCi/mM, New England Nuclear, recrystallized). An NADPH - generating system was not employed. After 12 to 24 hours of incubation, the medium was harvested and the

monolayer washed five times with 2 ml of 0.9% of NaCl. The pool of medium and washes was extracted four times with three volumes of ethyl acetate, after adding a known amount of testosterone - 1,2 - ^{3}H to estimate experimental losses. The extract was washed twice with 1/20 vol water, evaporated to dryness, and the residue was chromatographed on paper in Ligroin C: 75% methanol, 1:1, or Ligroin C: propylene glycol, and on silica gel G thin layer plates in chloroform: methanol, 98.5: 1.5, after adding 100 micrograms of authentic testosterone as a chromatographic standard. The radioactivity migrating with the mobility of testosterone was eluted in methanol and a fraction was added to 10 ml of scintillation fluid (11) for double label counting in a Packard Tri-Carb 3320 scintillation spectrometer at an efficiency of about 26% for ^{3}H and about 46% for ^{14}C. The radioactivity (dpm) of each isotope of testosterone in each sample was determined by automatic external standardization using a Wang 700 A computer programmed with calibration curves plotted from a set of quenched standards. The identity of the testosterone - 4 - ^{14}C recovered from the medium was confirmed by reverse isotopic dilution. The methods used for its recrystallization, as well as for the separation and recognition of its metabolites will be described in detail separately (9).

RESULTS

The rate of testosterone consumption from the medium by the various cell strains is shown in Table 1. From each patient, the strain derived from genital skin metabolised testosterone at a greater rate than the strain derived from nongenital skin. The increment varied from about 3 (RG, experiment 2) to 30 - fold (SJ, experiment 3). The results in experiments 5 and 6 were obtained on a strain derived from the prepuce of a newborn. The rate of

TABLE 1

THE RATE OF TESTOSTERONE METABOLISM BY VARIOUS CELL STRAINS DERIVED FROM GENITAL AND NONGENITAL SKIN

Expt.	Donor Age (yrs.)	Cell Strain[1]	In Vitro Age[2]	Protein per dish (mg)	Radioactivity recovered in testosterone / total radioactivity recovered (%)
1	21	RGL	24	1.34	11.4
		RGA	24	1.11	57.7
2	21	RGL	26	1.03	27.7
		RGA	26	1.07	76.1
3	11	ALF	16	0.78	12.4
		ALD	13	0.68	71.4
	21	SJF	20	0.82	2.7
		SJD	18	0.81	66.3
4	13	JLF	14	1.00	5.3
		JLD	14	1.03	79.9
	35	CRL	16	1.10	26.0
		CRA	15	0.91	66.9
5	1/52	ABF	21	0.79	2.4
6	1/52	ABF	36	0.73	8.9

1. The first two letters are the initials of the donor. The third letter defines the anatomic origin of the explant skin: L, labium majus; F, prepuce; D, deltoid area of the arm; A, abdomen.

2. The number of population doublings estimated to have been undergone by the cell strain at the time of the experiment.

testosterone metabolism by a foreskin cell strain does not appear to depend on the age of the donor. After having undergone comparable generations of cell division in vitro, the cultured fibroblasts of foreskin from an adult (SJ) metabolised testosterone as quickly as those from a newborn (ABF).

DISCUSSION

Our data reveal that in situ differences between human genital and nongenital skin in regard to their capacity to metabolise testosterone persist in their respective cell strains. To our knowledge, this is the first good illustration of the persistence in serially subcultured human diploid cell strains of a metabolic difference between nonmalignant somatic tissues of man. In order to fulfill Krooth's criteria (12) for accepting the heritability of this difference, it will be necessary to observe it in cloned cells derived from each anatomic source.

Differences have been noted in the replicative lifespan of diploid cell strains derived from different tissues of the same donor and from donors of different ages (13). The data we have obtained to date do not suggest that donor age or replicative age in vitro influence greatly the capacity of a cell strain to metabolise testosterone. Further observations on the possible influence of these two variables are planned.

These results also support the notion that patients with TF will display a mutant phenotype in fibroblast skin culture, and our preliminary observations in this regard are affirmative (14).

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