EVIDENCE FOR X-LINKAGE OF STEROID SULFATASE IN THE MOUSE: STEROID SULFATASE LEVELS IN OOCYTES OF *XX* AND *XO* MICE

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Manuscript received March 25, 1982
Revised copy accepted September 16, 1982

ABSTRACT

The steroid sulfatase (STS) levels in mature oocytes of *XX* and *XO* mice were assayed along with lactate dehydrogenase (LDH), an autosomal marker, and glucose-6-phosphate dehydrogenase (G6PD), a known X-linked gene. LDH levels in *XX* and *XO* oocytes were equal, whereas STS and G6PD levels were approximately twice as high in *XX* oocytes as in *XO* oocytes. These results indicate that the STS gene is X-linked in the mouse just as it is in humans. Assays of STS in kidney tissue of *XX* and *XO* mice indicated dosage compensation for the gene, which is different from that observed in humans.

IN most female mammals, dosage compensation for X-linked genes is achieved through inactivation of one of the *X* chromosomes in somatic cells. The mechanism is chromosomal, and the original X-inactivation hypothesis implied that one entire *X* chromosome was inactivated (LYON 1961). The clinical abnormalities characteristic of some human *X* chromosome aneuploidies did not seem compatible with the idea of the entire *X* chromosome in somatic cells being inactivated, and differences in phenotypes of individuals with short arm and long arm deletions of one *X* chromosome led to the hypothesis of a dosage-dependent region on the short arm of the human *X* chromosome (FERGUSON-SMITH 1965). Studies of the X_g^a gene, which is located on the short arm of the human *X*, suggest that this locus is not subject to X inactivation (RACE and SANGER 1975). Recently, studies of the steroid sulfatase (STS) gene, which is also on the short arm of the human *X* (SHAPIRO *et al.* 1979; MOHANDAS *et al.* 1979, 1980; MÜLLER *et al.* 1980a; TIEPOLO *et al.* 1980), have provided definitive evidence of noninactivation of this locus. Furthermore, dosage effects for STS expression in fibroblast cultures and hair follicles from one *X* and two *X* human individuals have been reported by several groups (MÜLLER *et al.* 1980b; ROPERS *et al.* 1981; CHANCE and GARTLER 1982). Thus, it is now established that one locus, and possibly a chromosomal region in humans, is not subject to X inactivation.

In the mouse there is no evidence for an X-linked locus or region that is not subject to X inactivation. Moreover, the *XO* female mouse is morphologically normal and fertile in contrast to the phenotypically abnormal and sterile human

XO female. Recently, it was reported that the STS locus in mice was autosomal (BALAZS *et al.* 1981). Until now all *X*-linked genes in one mammal have turned out to be X-linked in other mammals (OHNO 1973). The STS case would be the first instance of nonhomology of an *X*-linked locus in mammals. Because of the interest of this exceptional gene, we decided to investigate its possible *X* chromosomal linkage in the mouse by comparative germ cell studies in *XO* and *XX* mice. It has been demonstrated for several loci that both X chromosomes are active in meiotic stage germ cells (EPSTEIN 1969, 1972; GARTLER *et al.* 1972; GARTLER, LISKAY and GANT 1973; KOZAK, MCLEAN and EICHER 1974) in mice and humans, and comparisons of murine oocytes derived from *XX* vs. XO females have consistently yielded 2:1 ratios of activities for X-linked gene products. This report presents the results of our study of STS levels in mature germ cells and somatic tissues of *XO* and *XX* mice.

MATERIALS AND METHODS

Thirty-five *XO* mice and 33 normal female siblings (F_1s from C57BL/6J × CBA/Ca) were obtained from the Jackson Labs. The *XO* mice were hemizygous for the X-linked tabby (*Ta*) locus, whereas their normal female siblings were heterozygous (*Ta*/+) for the locus.

Mature follicle-clean oocytes were obtained by the method of ANDINA (1978). To get enough oocytes for STS assays, oocytes (from 33–118 in number) from two to four animals were pooled, washed in phosphate-buffered saline (PBS), counted, and placed in 10 μl of PBS. Sixty-five microliters of 0.1 M tris(hydroxymethyl)aminomethane (Tris), pH 7.2, buffer were added to the oocytes, followed by three freeze thawings in a dry ice-alcohol bath. The resulting homogenates were distributed into three portions: 50 μl for STS, 15 μl for glucose-6-phosphate dehydrogenase (G6PD), and 5 μl for lactate dehydrogenase (LDH) assays. G6PD and LDH were assayed according to the method of KRATZER and GARTLER (1978). All enzymatic determinations were carried out as paired assays (*XX vs. XO*) and, in the case of oocytes, performed immediately after their preparation. Kidneys from sacrificed animals were stored frozen at −70° and later disrupted in an Ultra-Turrax tissue homogenizer (Tekmar Company, Cincinnati, Ohio) in 1 ml of the above buffer. The homogenate was centrifuged at 150 × *g* for 10 min and the supernatant removed and freeze thawed three times in a dry ice-alcohol bath. Fifty microliters of this extract were used for STS assay, and 15 and 5 μl of a 1:20 dilution of the extract were used for G6PD and LDH assays, respectively. In addition, kidney extracts were also assayed for protein content by the Bio-Rad method (BRADFORD 1976).

STS activity was determined with ^{3}[H]-estrone sulfate as substrate (New England Nuclear, specific activity 59 Ci/mmol). ^{3}[H]-estrone sulfate (3.5 μCi) was mixed with the cell extract and 50 μl of 0.1 M Tris, pH 7.2, buffer and incubated at 37°. Samples (25 μl) of this mixture were withdrawn at 0-, 1-, and 2-h time points, and the product, ^{3}[H]-estrone, was isolated by thin layer chromatography on silica gel (Eastman Kodak, Rochester, New York) in 2,2,4-trimethyl pentane, ethyl acetate, butanol, methanol, 1 M ammonium hydroxide (1:2:1:2:1.5). Cold estrone was added to the chromatogram so that the position of the product could be detected upon exposure to iodine vapors. The ^{3}[H]-estrone spot was cut out and counted in toluene-Omnifluor in a liquid scintillation counter and the STS activity calculated as pmoles of ^{3}[H]-estrone formed per hour per oocyte.

RESULTS

Table 1 shows the results of the assays on oocytes from *XO* and *XX* mice. Simple inspection reveals that for LDH, *XO* and *XX* values are similar, whereas, for both G6PD and STS, *XX* values are generally greater than *XO* values. The means and standard errors of the means show identity of *XX* and *XO* means for LDH, but for G6PD and STS the *XX* means are approximately twice as great

TABLE 1

LDH, G6PD, and STS values for oocytes from XO and XX mice

Experiment	No. of oocytes		LDH (μmoles/hr/oocyte)		G6PD (nmoles/hr/oocyte)		STS (pmoles/hr/oocyte)	
	XO	XX	XO	XX	XO	XX	XO	XX
1	96	82	0.11	0.10	1.00	1.88	0.11	0.25
2	53	73	0.21	0.20	0.60	1.26	0.13	0.20
3	52	88	0.12	0.15	0.74	2.21	0.11	0.25
4	35	55	0.23	0.24		1.56	0.04	0.04
5	53	43	0.25	0.24	1.18	2.43	0.04	0.08
6	33	73	0.19	0.18		1.88	0.05	0.11
7	38	58	0.23	0.24		1.66	0.04	0.07
8	98	88	0.21		0.94	1.50	0.11	0.25
9	62	110	0.20	0.21	0.90	0.90	0.08	0.10
10	81	118	0.08	0.07	0.77	1.94	0.06	0.17
11	92	106	0.12	0.12	0.31	0.52	0.06	0.15
12	82	108	0.21	0.24	0.69	1.21	0.12	0.26
$\bar{X}$			0.18	0.18	0.79	1.58	0.08	0.16
S.E.M.			±0.02	±0.02	±0.09	±0.16	±0.01	±0.02

From 33 to 118 mature, follicle-clean, oocytes, from two to four animals, were pooled and freeze thawed in 75 μl of buffer for each set of determinations. For STS, 50 μl were used; for G6PD, 15 μl; and for LDH, 5 μl.

as the *XO* means. The *XX*:*XO* differences for G6PD and STS are highly significant even under a test in which the variances are assumed to be unequal (DIXON and MASSEY 1951) (t for G6PD is 3.96, $P < 0.01$; t for STS is 3.20, $P < 0.01$). Since these experiments were run as paired assays, a statistical analysis of the ratios of the paired assays is possible by converting the raw data to logarithms. This statistical method controls for possible extraneous factors and does not assume equality of the variances of the populations being tested (DIXON and MASSEY 1951). For LDH the mean of the paired ratios is 1.02, which does not differ significantly from the expected ratio of 1.00 for an autosomal locus ($t = -0.35$) but does differ significantly from 2.00 ($t = -22.6$), the value expected for an X-linked gene. For G6PD the mean is 1.95, which is significantly different from 1.00 ($t = -6.10$) but is not significantly different from 2.00 ($t = 0.62$), the value expected for an X-linked gene if both *X* chromosomes are active in oocytes. The same situation holds for STS (compared with 1.00, $t = -7.51$; compared with 2.00, $t = 0.41$).

As a control somatic tissue, we assayed kidney tissue from the same *XO* and *XX* mice used for the oocyte assays. The means and S.E.M.s for these assays are shown in Table 2, and, as can be seen, the means for the *XO*s and *XX*s are not significantly different from each other for any of the enzyme activities. The ratios of the mean *XX* to *XO* values are all very close to 1.00 (0.90, 1.06, and 1.04 for LDH, G6PD, and STS, respectively), which is the expected value for both autosomal and dosage compensated X-linked genes in somatic tissues of *XX* and *XO* mammals.

TABLE 2

LDH, G6PD, and STS values for kidney extracts from XO and XX mice

	LDH (μmoles/hr/mg protein)		G6PD (μmoles/hr/mg protein)	STS (pmoles/hr/mg protein)
	No.	$\bar{X}$ S.E.M.	$\bar{X}$ S.E.M.	$\bar{X}$ S.E.M.
XO	12	1.79 ± 0.15	0.17 ± 0.01	0.77 ± 0.07
XX	12	1.62 ± 0.16	0.18 ± 0.02	0.80 ± 0.11
τ value for *XO*:XX comparison		0.77	0.33	0.23

DISCUSSION

The 2:1 ratio of STS activity levels for germ cells in *XX* compared with *XO* mice indicates, but does not prove, that STS is X-linked in the mouse just as it is in humans. Such results are expected in view of the strong evidence for conservation of mammalian X-linkage (OHNO 1973). However, proof of X-linkage would require appropriate segregation data with an STS marker. BALAZS *et al.* (1981) have reported that C3H/An mice are STS deficient and that the deficiency segregates as if it were due to an autosomal recessive marker. If the STS gene turns out to be autosomal in the mouse, it would represent the first case of an X-linked gene in one mammal not being X-linked in other mammals. Although their data are not definitive, it is conceivable that the STS locus is autosomal in the mouse and that our results reflect some strange *X* chromosome-dependent effect at the level of STS activity in oocytes. It is also possible that in addition to an X-linked STS locus, there could be an autosomal locus affecting STS activity and that the C3H/An mutant is autosomal. Crosses with the scurfy mutant, the supposed analogue of human X-linked ichthyosis, could resolve this possibility. Our results under this latter case would still reflect a dosage effect of the X-linked STS locus. Yet another possibility with which the BALAZS *et al.* (1981) data are compatible is that STS is sex linked with alleles on both the *X* and Y chromosomes. A cross of an F_1 male, derived from a normal ♀ × deficient ♂, to a deficient female would distinguish XY linkage from simple autosomal recessive inheritance. XY linkage is not, to our knowledge, compatible with the human data on the genetics of STS deficiency.

Further work with the C3H/An STS deficiency is needed in order to clarify the genetics of STS in the mouse. However, it appears that the genetic control of STS in the mouse, with respect to either linkage or dosage compensation, may differ significantly from the STS system in humans.

This work was supported by Genetic Center grant GM15253 from the National Institute of General Medical Sciences. S.M.G. is the recipient of a Career Award from the National Institutes of Health.

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Corresponding editor: D. BENNETT