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Steroid Sulfatase Activities in Human Breast Tumors[1] (38109)

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The formation of various steroid sulfates by enzymes from human breast tumors has been reported (1, 2). These studies also suggest that the levels of the enzyme activities in the tumor, particularly those of dehydroepiandrosterone (DHEA) sulfotransferase and estradiol sulfotransferase can be correlated with the patient's response to bilateral adrenalectomy in the treatment of advanced cancer of the breast (3, 4). Thus, it was shown that patients whose breast cancers did not possess measurable enzyme activities for sulfurylation of DHEA and estradiol rarely responded to bilateral adrenalectomy, whereas breast tumors having the capacity to synthesize more DHEA sulfate than estradiol sulfate often regressed after adrenalectomy. Since endogenous sulfatases may influence the apparent level of sulfate formation in the breast tumors, it seems important to further study steroid sulfatases in human breast cancers in relation to the enzyme activity catalyzing the formation of steroid sulfates.

Materials and Methods. 7α-^{3}H-DHEA sulfate (2 Ci/mmole), 6,7-^{3}H-estrone sulfate (470 mCi/mmole), and 7α-^{3}H-testosterone sulfate (2 Ci/mmole) were purchased from Amersham/Searle.

Immediately after surgical excision, tumors were minced with scissors, and homogenized in an all-glass Potter–Elvehjem homogenizer with ca. 4 vol of 0.25 *M* sucrose. The homogenate was centrifuged for 10 min at 2000 rpm (Sorvall SS-34 rotor) and the supernatant was used as the source of enzyme.

The method of Burstein and Dorfman (5) was used to assay for steroid sulfatase activity when either DHEA sulfate or testosterone sulfate was the substrate. When estrone sulfate was the substrate, the assay was carried out in the same way except that no NaOH was added before the extraction into toluene. Assays were carried out at two enzyme concentrations and reactions were stopped at 0, 30, and 60 minutes of incubation at 37°. Control experiments with boiled enzymes were regularly carried out. Protein determinations were carried out by the biuret method. Results were calculated as pmoles hydrolyzed/mg protein/hr.

Results. In this study, the ability of human breast tumor preparations to hydrolyze three steroid sulfates was examined. The sulfates used as substrates were DHEA sulfate (a 3β-yl sulfate), estrogen sulfate (a phenyl sulfate), and testosterone sulfate (a 17β-yl sulfate). In agreement with the substrate specificities of other mammalian steroid sulfatases reported by Roy (6), we found that testosterone sulfate was not hydrolyzed by these preparations.

Tables I and II present data on steroid sulfatase activities in 16 primary and 15 metastatic breast tumors. The primary tumors were those removed from the breast at the time of mastectomy. The metastatic tumors were soft-tissue metastatic lesions in the subcutaneous tissues or lymph nodes. They were all confirmed histologically as adenocarcinomas. All of these preparations contained an estrogen sulfatase activity (or possibly an aryl sulfatase activity), but only nine of the primary tumor preparations, and 10 of the metastatic tumors also exhibited sulfatase activity toward DHEA sulfate. In all cases, the DHEA sulfatase activity was much lower than the estrogen sulfatase activity.

Since it is possible that the sulfatase activi-

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TABLE I. Steroid Sulfatase Activity and Steroid Conjugation in Primary Breast Cancer Tumors.

	Sulfatase activity (pmoles/mg protein/hr)		Steroid sulfate synthesis (pmoles/mg protein/hr)		
Patients	E_1S[a]	DHEAS[b]	E_2[c]	DHEA	Ratio DHEA/E_2
L.A	56.0	1.2	0	0	0
O.B.	1.7	0	44.0	65.0	1.5
O.B.	9.8	0.5	0	0	0
E.D.	1.1	0	30.7	29.4	0.9
R.E.	12.0	0.3	54.5	28.2	0.5
I.F.	80.0	0.4	92.5	176.7	1.9
G.G.	3.5	0	0	0	0
C.H.	6.2	0	16.8	14.0	0.8
T.H.	12.2	0	3.1	2.4	0.8
V.N.	4.9	0	17.4	72.5	4.2
B.P.	4.7	0.3	19.4	13.8	0.7
A.P.	48.9	3.4	7.0	9.6	1.4
M.R.	11.9	0.6	62.9	49.6	0.8
R.S.	12.8	0.9	8.4	7.8	0.9
F.S.	9.7	0.5	28.6	39.7	1.4
A.V.	10.1	0	9.9	43.5	4.4

[a] E_1 = Estrone sulfate.
[b] DHEA = Dehydroepiandrosterone sulfate.
[c] E_2 = Estradiol.

ties of breast tumor preparations might affect the conjugation of steroids, the sulfotransferase activity in these same tumor preparations was simultaneously examined. The re

TABLE II. Steroid Sulfatase Activity and Steroid Conjugation in Metastatic Breast Lesions.

	Sulfatase activity (pmoles/mg protein/hr)		Steroid Sulfate Synthesis (pmoles/mg protein/hr)		
Patients	E_1S[a]	DHEAS[b]	E_2[c]	DHEA	Ratio DHEA/E_2
L.D.	3.1	0.3	9.8	8.2	0.8
E.M.	5.2	0.9	19.5	27.8	1.4
I.P.	4.1	0	6.8	8.0	1.2
E.S.	2.8	0	28.1	21.4	0.8
F.S.	6.6	0.4	0	0	0
M.S.	14.3	0.7	38.1	46.7	1.2
J.W.	16.5	6.0	10.1	39.8	3.9
L.G.	7.4	0	21.2	24.4	1.1
J.J.	4.6	0.3	25.1	26.3	1.0
E.O.	12.8	0.6	6.3	7.8	1.2
O.B.	5.6	0.5	163.2	310.8	1.9
M.P.	5.5	0	8.1	31.3	3.9
E.B.	7.2	0	5.3	6.7	1.3
M.P.	6.4	0.3	48.0	27.0	0.5
P.R.	1.8	0.3	8.1	7.9	0.9

[a] E_1S = Estrone sulfate.
[b] DHEA = Dehydroepiandrosterone sulfate.
[c] E_2 = Estradiol.

TABLE III. Steroid Conjugation by Breast Tumors with No Sulfatase Activity.

	Steroid conjugation activity			
	No. of tumors	No activity	DHEAS/E_2S[a] > 1	DHEAS/E_2S < 1
Primary tumors	29	6	16	7
Metastatic tumors	25	7	6	12
Total	54	13	22	19

[a] DHEAS = Dehydroepiandrosterone sulfate; E_2S = Estradiol sulfate.

sults shown in Tables I and II do not suggest that levels of sulfatase activity affect those of sulfotransferase activity. On the contrary, it appears that sulfatase activity is more likely to be present in tumors with sulfotransferase activity, since some 87% of the tumors with sulfatase activity also have sulfotransferase activity.

Table III presents the results of steroid sulfotransferase activity of some 54 breast tumor preparations containing no sulfatase activity. Again, there is no correlation between the lack of sulfatase activity and the presence or absence of sulfotransferase activity. In this series, 24% show no steroid sulfotransferase activity, 41% show a ratio of DHEAS to E_2S greater than 1 and 35% show a ratio less than one, a distribution which is quite similar to the overall distribution we have found over the past several years.

Discussion. The results reported in this paper clearly demonstrate that there is no relation between sulfatase activity and either the presence or the absence of sulfotransferase activity in the mammary tumors. The data also show that the ratio of sulfotransferase activity with DHEA and estradiol as substrates is not related to the sulfatase activity in these tumor preparations.

It must be pointed out that in the present study we are measuring the activity of estrone sulfatase, whereas in our conjugation (sulfotransferase) studies, we measure the formation of estradiol-3-sulfate. This, however, will not invalidate our conclusions, for two reasons, first, estrone is nearly as good a substrate as estradiol for the human tumor sulfotransferase enzyme (3). Second, we believe that the presence of a β hydroxyl group, rather than a ketone group at the 17 position would make little, if any, difference to the sulfatase enzyme activity.

In the present study, we find that the levels of estrone sulfatase are consistently much higher than the levels of DHEA sulfatase activity. Indeed, of the 31 preparations with estrogen sulfatase activity, only 19 (61%) show any DHEA sulfatase activity, and in all cases except 1 (J.W.) the estrogen sulfatase activity is approximately 10-fold or more higher than DHEA sulfatase activity. The lack of DHEA sulfatase activity in the remaining 39% of the tumor preparations with estrogen sulfatase is even more striking when one considers that the DHEA sulfate used for the assay has approximately four times the specific activity of the estrone sulfate used for the estrogen sulfate assay. It should also be noted that this considerably higher level of estrone sulfatase activity in the tumor preparations does not seem to influence the ratios of DHEA sulfate to estradiol sulfate.

Two possible explanations for this great disparity between estrogen sulfatase and DHEA sulfatase activity are considered. First, we may not be measuring a true estrogen sulfatase, but one of the ubiquitous aryl sulfatases (7). Second, recent studies (8) have suggested that DHEA sulfate may be the preferred precursor for the biosynthesis of estrogens. Since breast tumors have been shown to convert C-19 compounds to estrogens (9, 10), it is conceivable that in these tissues DHEAS is also the preferred precursor, and thus the presence of a DHEA sulfatase would hinder estrogen synthesis. However, since estrogen is hormonally active as the free steroid, it would be useful to have an estrogen sulfatase to cleave the phenol-sulfate bond once the aromatization has taken place. At present it cannot be determined if either one of these possibilities is correct.

Summary. The ability of human breast tumor preparations to hydrolyze DHEA, estrone,

and testosterone sulfates was studied. Of the 85 tumor preparations examined, 31 tumors contained an estrogen sulfatase activity and 19 of which also exhibited sulfatase activity toward DHEA; but there was no testosterone sulfatase in any of the 31 tumor preparations. The remaining 54 tumor preparations showed no sulfatase activity toward any of the three steroid sulfates examined. The present data also disclose that the levels of sulfatase activities do not affect those of sulfotransferase activities in the tumor preparations. Further, the ratio of sulfotransferase activity with DHEA and estradiol as substrates is not related to the sulfatase activity in these tumor preparations.

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