Effect of the stress of unilateral adrenalectomy on the depletion of individual cholesteryl esters in the rat adrenal

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ABSTRACT Normal rats were subjected to unilateral adrenalectomy and were killed 3 hr later. The concentration and composition of the cholesteryl esters in adrenals removed at operation and after death were compared. The esterified cholesterol concentration was lower in the adrenals obtained 3 hr after surgery. Cholesteryl arachidonate decreased in concentration significantly more than any other ester, followed in order of magnitude by linoleate and oleate. The cholesteryl ester concentration of adrenals removed from sham-operated rats 3 hr after surgery was also greatly reduced.

On the basis of comparison with other work on the hydrolysis of cholesteryl esters by adrenal homogenates, it is concluded that the apparent selectivity in depletion of cholesteryl esters is due to differences in their rates of hydrolysis.

SUPPLEMENTARY KEY WORDS cholesteryl arachidonate · adrenate · linoleate · oleate · preferential hydrolysis

Most of the cholesterol in rat adrenal glands, in contrast to that in other tissues, is esterified; the esterified fatty acids characteristically contain rather high percentages of the tetraenoic acids, arachidonic and adrenic (7,10,13,16-docosatetraenoic) (1–3). Sayers, Sayers, White and Long (4) showed that administration of ACTH to rats caused a depletion of cholesterol in the adrenal. Popják (5) showed a depletion of adrenal cholesteryl esters in rats stressed by a crushing injury. The present study determined whether there is a selective depletion of cholesteryl esters of the rat adrenal, especially esters of polyunsaturated acids, when the total cholesteryl ester concentration decreases as a result of the stress of unilateral adrenalectomy.

MATERIALS AND METHODS

Male Sprague–Dawley rats, weighing 480–600 g, were maintained on mouse pellets (Rockland Farms, New City, New York).

The rats were anesthetized with ether and unilaterally adrenalectomized. The "control" adrenal was removed through a midventral incision which was closed with skin clips. The entire surgical procedure lasted 3–6 min. In five rats the left adrenal was excised and in five others the right. Subsequent analyses revealed no differences between right and left adrenals with regard to concentration or composition of cholesteryl esters. 3 hr after the operation the rats were killed and the second (stressed) adrenal was removed. The glands were trimmed of surrounding fat and tissue, weighed, and kept in ice-cold Ringer's injection solution (Abbott Laboratories, North Chicago, Illinois) until homogenization. The mean weights of the adrenals were 24.3 (20.2–26.0) mg for the control and 25.6 (22.0–30.2) mg for the stressed adrenals. These weights expressed per 100 g body weight were 4.8 (4.5–5.1) mg and 4.9 (4.5–5.8) mg, respectively.

Individual adrenals were homogenized in a small volume of chloroform–methanol 2:1. The homogenate was filtered through glass wool, and the volume of the filtrate was adjusted to 10 ml with the same solvent mixture. Aliquots were taken for analyses of free and

Abbreviations: ACTH, adrenocorticotrophic hormone; GLC, gas–liquid chromatography.

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total cholesterol (6). The remainder of the lipid extract was combined with the supernatant solution obtained after the precipitation of cholesterol digitonide (6) and dried in vacuo, and the residue was extracted with a small volume of ether. From this solution cholesteryl esters were isolated by thin-layer chromatography in hexane–diethyl ether–acetic acid 83:16:1. Methyl esters of the cholesteryl ester fatty acids were prepared, purified, and analyzed by gas chromatography as described previously (3, 7).

In a second series of experiments, male Sprague–Dawley rats, weighing 305–395 g and maintained on mouse pellets, were used to study the effect of sham operation on the concentration of adrenal cholesteryl esters.

Cholesterol analyses (as described above) were performed on adrenals from rats comprising the following three groups: (*a*) animals killed by decapitation or over-anesthetization, (*b*) animals killed 3 hr after sham operation, and (*c*) animals which were unilaterally adrenalectomized and killed 3 hr later. The mean weights of the adrenals were 21.4 (17.2–26.6) mg for the control glands (*a* and *c*) and 19.7 (17.6–24.0) mg for the stressed adrenals (*b* and *c*). These weights expressed per 100 g body weight were 6.4 (5.2–7.5) mg and 5.6 (4.8–6.3) mg, respectively. The fatty acid composition of the cholesteryl esters of these adrenals was not determined.

RESULTS

Table 1 shows the free and esterified cholesterol concentrations in the control and stressed adrenals. The drop in concentration of esterified cholesterol was highly significant, whereas the concentrations of free cholesterol in the adrenals did not change significantly.

The effect of sham operation on the cholesteryl ester concentrations of the adrenals is shown in Table 2. There were no differences between left and right adrenals removed at the same time from the same type of animal, whether normal or sham operated. The data indicate that sham operation by itself produced sufficient stress to reduce the cholesteryl ester concentrations 3 hr later. Differences between any group of control and stressed adrenals were highly significant ($P < 0.005$ or $P < 0.001$). Free cholesterol concentration was unaffected by stress, the values being 2.6 ± 0.1 and 2.5 ± 0.1 mg/g for the control and stressed adrenals, respectively.

The fatty acid composition of adrenal cholesteryl esters is shown in Table 3. Palmitate predominates and comprises nearly 29% of the total esters. The three other major esters are oleate, arachidonate, and adrenate. After adrenalectomy, percentages of myristate and adrenate were higher and those of oleate, linoleate, and arachidonate were lower. The only statistically significant decrease was in the percentage of arachidonate.

In order to determine the contribution of each cholesteryl ester to the total decrease of cholesteryl esters, we calculated molar concentrations of the individual esters by multiplying the GLC value by the

TABLE 1 CHOLESTEROL CONCENTRATIONS IN CONTROL AND STRESSED ADRENAL GLANDS

Cholesterol	Control	Stressed	Mean of Differences between Pairs	*P*
		mg/g (wet weight)		
Free	4.2 ± 0.4	3.4 ± 0.2	0.8 ± 0.5	<0.2
Esterified	44.0 ± 1.9	25.6 ± 2.0	18.4 ± 1.2	<0.001

The analyses were performed on adrenals from 10 rats. The values are the means ± SEM.

TABLE 2 CHOLESTERYL ESTER CONCENTRATION IN CONTROL AND STRESSED ADRENAL GLANDS

	Left Adrenal		Right Adrenal
		mg/g (wet weight)	
Control	(6)*	42.2 ± 2.5	39.7 ± 3.3
Sham operated	(5)	15.8 ± 3.8	16.9 ± 3.8
Adrenalectomized†	(5)	36.1 ± 3.1	14.6 ± 1.2
All control	(17)	39.5 ± 1.7	
All stressed	(15)	15.8 ± 1.7	

Values are means ± SEM.
* Number of left or right adrenal glands analyzed.
† In this group, the left adrenal was the control (see text).

TABLE 3 FATTY ACID COMPOSITION OF ADRENAL CHOLESTERYL ESTERS

Fatty Acid	Control	Stressed
	moles %	
12:0	tr.	tr.
14:0	3.1 ± 0.3	5.8 ± 0.4*
15:0	0.5 ± 0.1	0.6 ± 0.1
16:0	28.7 ± 1.9	31.9 ± 1.3
16:1	3.6 ± 0.4	4.4 ± 0.4
18:0	3.8 ± 0.2	4.4 ± 0.3
18:1	18.2 ± 1.1	14.0 ± 1.6†
18:2	3.7 ± 0.3	2.9 ± 0.3
20:0	1.2 ± 0.1	1.5 ± 0.1
20:1	2.3 ± 0.1	2.6 ± 0.2
20:2	0.5 ± 0.0	0.4 ± 0.1
20:3	1.2 ± 0.1	1.4 ± 0.3
20:4	13.6 ± 0.5	6.2 ± 0.7‡
20:5	0.8 ± 0.1	0.9 ± 0.2
22:1	0.9 ± 0.1	1.5 ± 0.3
22:3	—	—
22:4	13.4 ± 0.3	16.0 ± 1.3†
22:5	1.5 ± 0.2	1.5 ± 0.2
22:6	2.4 ± 0.3	3.0 ± 0.3
24:1	0.8 ± 0.2	1.2 ± 0.3

Fatty acids are designated by number of carbon atoms:number of double bonds. Values are means ± SEM (n = 7 pairs of adrenals).
* $P < 0.005$.
† $P < 0.1$.
‡ $P < 0.001$.

molar concentration of esterified cholesterol for each adrenal. Table 4 shows the absolute changes in tissue concentrations for each ester.

Values for the decrease of each ester as a percentage of the total decrease are in column 5 of Table 4. Approximately 77% of the total decrease can be accounted for by decreases in palmitate, oleate, and arachidonate. If there were no selectivity with regard to cholesteryl ester decreases as a result of stress, the percentage contribution of a given ester to the total decrease should be identical with its percentage of the total esters in the control adrenal (column 6 of Table 4, data from Table 3). The ratio of percentages in column 5 to those in column 6 is an "index of selectivity" with regard to cholesteryl ester decreases (depletion). These ratios are in column 7. Values greater than unity suggest selective loss of an ester. Cholesteryl oleate, linoleate, and especially arachidonate are depleted to a greater extent than esters of other acids.

From the concentration of an ester in the control adrenals (Table 4, column 2) and the difference between the concentrations in the control and stressed adrenals (Table 4, column 4) one can calculate the percentage decrease for each ester. The mean decrease of total esters for these seven pairs of adrenals was 38.0 ± 2.3%. The percentage decreases for individual esters were 29.7 ± 5.2 for palmitate, 49.0 ± 9.5 for oleate, 48.0 ± 8.6 for linoleate, 72.8 ± 3.6 ($P < 0.001$) for arachidonate, and 27.7 ± 5.9 for adrenate.

DISCUSSION

This study shows that there is a large decrease in the concentration of adrenal cholesteryl esters 3 hr after unilateral adrenalectomy or sham operation and that free cholesterol levels are unchanged. The data thus are in accord with the observations of others who have demonstrated that similar changes occur in many species as a result of stress or ACTH administration (8). The increased steroid hormone synthesis and secretion which also occur have led to the speculation that cholesteryl esters may be precursors of adrenocortical steroids. Sinclair has suggested that the polyunsaturated acids of the adrenals, ovaries, and testes may be involved in the synthesis or metabolism of steroid hormones (9). The fact that a large proportion of adrenal cholesteryl esters contain such acids makes one wonder whether such polyunsaturated esters may play an important role in steroid biogenesis.

One approach to the study of selective utilization of cholesteryl esters would be to estimate the turnover of the individual esters before and after stimulation with ACTH. A second approach is that undertaken in the present study, i.e., to determine whether the depletion of cholesteryl esters in a stimulated adrenal is selective. Dailey, Swell, Field, and Treadwell have reported (1) that there may be changes in the proportions of arachidonic and docosatetraenoic acids in rat adrenal cholesteryl esters after administration of ACTH, but they stated neither the magnitude nor direction of the changes.

The analyses in Table 3 show that the proportion of several esters change as a result of stress. Most notably the proportions of arachidonate and oleate are decreased, while there are increases in palmitate and adrenate. There were some changes in minor esters, but only the increase in myristate was of statistical significance. The values in Table 4 show that there were

TABLE 4 CONCENTRATIONS OF CHOLESTERYL ESTERS IN CONTROL AND STRESSED ADRENALS

Ester (1)	Control (2)	Stressed (3)	Mean of Differences between Pairs (4)	Percentage of Total Decrease (5)	Moles % (Control)* (6)	(5)/(6) (7)
		μmoles/g (wet weight)				
14:0	3.6 ± 0.5	4.1 ± 0.2	+ 0.4 ± 0.5	—	3.1	—
16:0	33.0 ± 2.3	23.0 ± 2.0	−10.0 ± 2.0	22.6	28.7	0.8
16:1	4.2 ± 0.6	3.1 ± 0.2	− 1.1 ± 0.3	2.5	3.6	0.7
18:0	4.3 ± 0.2	3.2 ± 0.4	− 1.1 ± 0.5	2.5	3.8	0.7
18:1	21.1 ± 2.0	10.4 ± 1.9	−10.7 ± 2.4	24.2	18.2	1.3
18:2	4.3 ± 0.4	2.1 ± 0.3	− 2.2 ± 0.5	5.0	3.7	1.4
20:1	2.6 ± 0.2	1.8 ± 0.2	− 0.8 ± 0.2	1.8	2.3	0.8
20:4	15.8 ± 1.0	4.4 ± 0.4	−11.4 ± 0.9	25.8	13.6	1.9
22:4	15.5 ± 1.0	11.4 ± 1.1	− 4.1 ± 1.2	9.3	13.4	0.7
22.6	2.7 ± 0.3	2.2 ± 0.3	− 0.5 ± 0.4	1.1	2.4	0.5
Total†	111.8	67.6	−44.2‡			

Values are means ± SEM (n = 7 pairs of adrenals).
* From Table 3.
† These values do not include 14:0 data.
‡ This value includes the decreases observed for esters of 15:0, 20:0, 20:3, 20:5, 22:1, 22:5, and 24:1. The decrease in concentration of these esters was 2.3 μmoles/g, or 5.2% of the total decrease. The mean concentration of any of these esters never exceeded 1.7 μmoles/g and 1.1 μmoles/g in control and stressed adrenals, respectively.

decreases in absolute concentrations of all esters except myristate. The decreases in arachidonate and, to a lesser extent, in oleate and linoleate were selective. Although the fatty acid compositions of the cholesteryl esters of adrenals from sham-operated animals were not determined, it is likely that they would be similar to those of the stressed adrenals in Table 3. This presupposes a common mechanism by which cholesteryl esters are depleted in stressed adrenals irrespective of the manner in which stress is induced.

Riley (10) has suggested that cholesteryl esters may serve as immediate precursors of hormonal steroids. However, work of Dailey, Swell, and Treadwell (11) with hog adrenal homogenates has shown that radioactive steroids are made from radiolabeled cholesterol but not from cholesterol-labeled cholesteryl esters. Moreover, the hydrolysis of cholesteryl esters by hog adrenal homogenates could not be demonstrated. In later studies with canine adrenal homogenates, however, the same investigators demonstrated the hydrolysis of cholesteryl esters and the synthesis of labeled steroids from labeled cholesteryl esters (12). Thus, it would appear that hydrolysis of esters must precede the conversion of the sterol into steroids, and that cholesteryl esters can act as a reservoir of steroid precursors. Lipid droplets of the adrenal are depleted after administration of ACTH (13). Recently Moses, Davis, Rosenthal, and Garren (14) have demonstrated that 70–80% of adrenal cholesterol is located in these droplets. Thus, just as mobilization of lipid from fat cells of adipose tissue is stimulated by ACTH, cholesterol seems to be mobilized from fat cells of the adrenal when steroidogenesis is stimulated. In each situation mobilization is associated with hydrolysis. It is not known, however, whether cholesteryl esters are hydrolyzed before or after they are mobilized from the adrenal fat droplets.

Skovsted, Funch, and Dam (15) reported that stimulation of rat adrenals in vivo by administration of ACTH did not affect the amount or composition of polyunsaturated fatty acids of total adrenal lipids. Riley (10) has demonstrated an increased amount of phospholipid in the stimulated adrenal. It is conceivable that new phospholipid may be synthesized from fatty acids released as a result of cholesteryl ester hydrolysis. Such a sequence of reactions would account for the constancy of polyunsaturation in the total lipids in the adrenal.

Although the present study provides no information concerning the metabolic fate of the cholesteryl esters, one can assume on the basis of the above work that they were hydrolyzed and that the cholesterol was subsequently converted to hormonal steroids and hormonal steroid precursors. Differences in the ratio (5)/(6) of Table 4 would then reflect differences in the rates of hydrolysis of individual cholesteryl esters. Such differences have been reported. Dailey et al. (12) observed that the hydrolysis of cholesteryl palmitate by canine adrenal homogenates was less than that of unsaturated esters. The difference between arachidonate and adrenate is of interest in view of the facts that both are tetraenoic acids, and adrenate is unique to the adrenal (16). The difference in the degree of decrease of each of these esters probably reflects the marked specificity of the hydrolase with respect to chain length of the fatty acid and (or) the positions of the double bonds in relation to the ester group. The relatively large decrease of cholesteryl arachidonate may represent a selective utilization of this ester only inasmuch as it is hydrolyzed at a more rapid rate than other esters.

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