DECREASED AVAILABILITY OF HEPATIC NADPH IN STRESSED MICE

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Summary. Incorporation of ^{3}H-mevalonic acid into cholesterol is significantly impaired in liver homogenates from two inbred strains of mice when the livers are taken from mildly stressed animals. Addition of a NADPH-generating system to the homogenates reverses the stress effect almost completely in the C57BL/10 strain but only partially in the DBA/2 strain. The effect is temporally correlated with the adrenal response to the stress.

We have recently reported that of the two inbred mouse strains, C57BL/10 and DBA/2, liver homogenates from the former strain show significantly greater availability of endogenous NADPH, both for corticosterone metabolism (1) and for the biosynthesis of cholesterol from mevalonic acid (MVA) (2). These studies led us to suspect an acute effect of stress upon availability of NADPH since maximal interstrain differences were only obtained when the animals were killed in the animal room within a few minutes of the experimenter's entrance. A specific effect of stress upon hepatic NADPH availability does not appear to have been documented previously. We therefore describe definitive experiments which demonstrate such an effect in two strains of mice using as an indicator of hepatic NADPH availability the efficiency of metabolism of ^{3}H-mevalonic acid to sterols (2).

Male C57BL/10 and DBA/2 mice were either purchased from Jackson Memorial Laboratory, Bar Harbor, Maine when 5-6 weeks old, then maintained in our laboratory for at least three weeks before use, or were bred in our laboratory

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Table 1. Incorporation of ^{3}H-mevalonic acid into cholesterol and its precursors in homogenates of stressed and unstressed mice of the C57BL/10 and DBA/2 strains. Results are pooled from three experiments; n=7 for each group. Values are given ± SEM where applicable. The symbols + and - attached to the strain designations indicate addition or omission of a NADPH-generating system respectively. U indicates unstressed animals; S indicates stressed animals.

Fraction[1]	C57BL/10+		C57BL/10-		DBA/2+		DBA/2-	
	U	S	U	S	U	S	U	S
^{3}H in non-saponifiable material (As percent available ^{3}H-MVA)	17.8 ±0.8	21.6 ±1.5	9.9 ±1.8	8.6 ±0.7	21.7 ±1.5	19.3 ±2.2	7.6 ±0.7	8.0 ±1.3
^{3}H in chromatographic fractions (Percent total non-saponifiable ^{3}H)								
Fraction A: Squalene and squalene 2,3-oxide	4.3 ±0.6	7.2 ±1.2	8.1 ±2.3	20.8 ±4.2	5.4 ±0.6	14.1 ±2.7	12.5 ±3.4	19.8 ±2.8
Fraction B: Presqualene alcohol, lanosterol and related sterols	3.9 ±0.7	5.7 ±0.7	10.3 ±2.4	16.1 ±1.8	4.5 ±0.5	11.0 ±3.1	25.6 ±2.2	34.2 ±1.1
Fraction C: C_{27} sterols	66.3 ±3.0	58.9 ±3.6	55.3 ±8.4	25.5 ±4.6	64.0 ±2.5	42.4 ±6.0	27.5 ±2.5	15.0 ±1.4
Cholesterol from fraction C purified via dibromide								
as percent of non-saponifiable	45.9	36.6	35.2	13.5	43.6	29.0	12.4	6.1
as percent of available MVA	8.2	7.9	3.5	1.2	9.5	5.6	0.94	0.49
Ratio of cholesterol synthesized[2] Unstressed/Stressed	1.04		2.92		1.70		1.92	

1 For details of isolation of fractions see Gaskin and Clayton (1972). Other unidentified materials besides the major components indicated may be present in fractions A and B.

2 Calculated on the basis of the percent incorporation of available ^{3}H-MVA cholesterol.

from similar purchased breeding stock. The mice were strictly undisturbed for 36 hr. before the experiment. Unstressed mice were killed within ca. 3 min. of the experimenter's entering the animal room. Half the mice in one cage were unstressed controls; the other half were stressed. (There was no evidence that the metabolism of MVA depended upon time of sacrifice within the

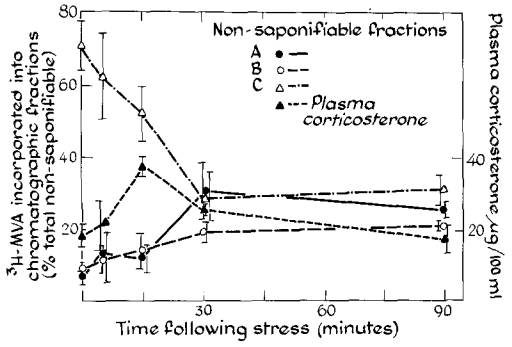

Fig. 1. Time course of effect of stress upon incorporation of ^{3}H-MVA into non-saponifiable materials by homogenates of C57BL/10 mouse liver, correlated with plasma corticosterone concentration. Each point gives the mean ± SEM for four mice. Incubations were performed (2) without the addition of a NADPH-generating system. For descriptions of fractions A, B and C, see Table 1. The percentage incorporations of ^{3}H-MVA into non-saponifiable material fell from 11.9 ± 1.9 at 0 min. to 8.3 ± 0.6 at 90 min.

experimental period, 9-11 a.m.) To induce stress, mice (Table 1) were put into a new cage in groups of 2 or 3, then moved to a new room for 40 min. before sacrifice. Alternatively, in the study shown in Fig. 1, mice were injected i.p. with 100 µl physiological saline and put singly into a new cage for the specified time before sacrifice. The mice were decapitated, blood was collected in heparinized centrifuge tubes and later assayed for plasma corticosterone concentration (3). Corticosterone concentrations (µg/100 ml ± S.E.M.) in plasma collected from each animal at the time of sacrifice (Table 1), were 12.8 ± 1.3 and 22.7 ± 3.6 for unstressed and stressed C57BL/10 respectively, and 19.7 ± 2.5 and 30.4 ± 3.6 for unstressed and stressed DBA/2 respectively.

The livers were rapidly chilled in ice-cold .08 M phosphate buffer pH 7.4 then individually homogenized (2.5 ml/gram liver) (4) and centrifuged for 10 min. at 600 xg. Procedures for 3 hr. incubations of 300 µg ^{3}H-MVA (38 x 10^6 dpm) in 1 ml homogenate, extraction of the non-saponifiable material and its

analysis by TLC, and radioassay methods have been described (2). The NADPH-generating system contained 2 μmoles NADP, 6 μmoles glucose-6-phosphate and 4 units glucose-6-phosphate dehydrogenase. The fractions containing ^{3}H-labeled, biosynthetic cholesterol were pooled for similar experimental groups and diluted with unlabeled cholesterol which was then purified via the dibromide (5) and radioassayed. The percent ^{3}H-cholesterol in the biologically labeled material was calculated.

The results (Table 1) confirm our earlier findings (2). With no NADPH-generating system added to the homogenates much less ^{3}H-MVA enters the C_{27} (cholesterol-containing) fraction C in the DBA/2 than in the C57BL/10 homogenate, but more ^{3}H-MVA enters the precursor fractions in the former strain. The addition of the NADPH-generating system essentially eliminates these interstrain differences.

The incorporation of ^{3}H into fraction C and into purified cholesterol is significantly lowered ($p < 0.05$) by stress in liver homogenates of both strains when no NADPH-generating system is added. Addition of the NADPH-generating system reverses these effects almost completely in the C57 strain but in the DBA strain the differences induced by stress remain significant ($p < 0.05$). Labeled precursors of cholesterol (fractions A and B), which require several equivalents of NADPH for their further metabolism, accumulate in the homogenates from stressed animals and this effect is counteracted when the NADPH-generating system is added. These results are most simply interpreted to indicate a decreased availability of endogenous NADPH following acute stress. Interestingly, the C57BL/6 strain of mice gave results closely similar to those obtained with the related C57BL/10 strain (Gaskin and Clayton, unpublished work).

The stress effect on NADPH in C57BL/10 develops synchronously with the rise in plasma corticosterone concentration but persists much longer (Fig. 1). Whether it depends upon the adrenal requires further investigation. Repeated additions of glucose-6-phosphate to the homogenates from stressed C57BL/10 animals during incubation did not improve their NADPH supply. A mechanism

based on adrenal-independent stimulation of glucose-6-phosphatase (6) therefore seems unlikely. Neither the soluble nor microsomal forms of glucose-6-phosphate dehydrogenase, nor 6-phosphogluconate dehydrogenase, was detectably inhibited in liver homogenates of C57BL/10 animals whose hepatic NADPH supply was curtailed and plasma corticosterone levels elevated by stress (7). These findings do not support the proposed inhibitory role of free fatty acids (8,9).

The strain difference in the results (Table 1) suggests the interesting possibility that stress may stimulate NADPH-utilization by a pathway which in the DBA/2 is relatively more important than in the C57 strain. A recent report (10) that the biosynthesis of fatty acids, sterols and proteins is inhibited in rat liver slices incubated with cyclic AMP possibly relates to our findings.

Our results may help to explain the poorly understood inhibition of *in vivo* metabolism of steroids under conditions of acute stress (11). This possibility is being investigated further.

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