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THE EFFECTS OF SKF 525-A ON HEPATIC GLYCOGEN AND RATE OF HEPATIC DRUG METABOLISM*

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Abstract—The level of hepatic glycogen and rates of certain drug metabolisms were studied after administration of SKF 525-A (β-diethylaminoethyl diphenylpropyl acetate HCl) to adult rats. SKF 525-A inhibits hepatic drug metabolism within 1 hr, and a significant effect is seen up to 24 hr after administration. SKF 525-A lowers hepatic glycogen to a minimum level in 8 to 12 hr. The recovery of hepatic glycogen and rate of hepatic drug metabolism to control values occurs at the same time after administration of SKF 525-A. The effects of SKF 525-A on certain enzymes of hepatic glycogenesis and glycogenolysis were also studied.

A POSSIBLE relationship between structure and certain functions of the hepatic endoplasmic reticulum has been suggested.[1] Fouts has shown that the hepatic enzymes which metabolize certain foreign substances are located predominantly in the smooth-surfaced endoplasmic reticulum (SER).[2] Porter and Bruni have shown an association between hepatic glycogen deposits and the SER.[3] These latter authors postulated a possible "glycogen storage or release" function of the SER based on changes in electron microscopic appearance of this structure during fasting and feeding experiments.[4]

Physiological factors which alter hepatic glycogen levels often affect the enzymic metabolism of foreign substances by the SER. Such factors include starvation,[5] alloxan diabetes,[6] and epinephrine administration.[7] Aminoazo-carcinogens destroy the ability of the hepatic cell to store glycogen,[3, 8] and hepatic tumors have been shown to be deficient in drug-metabolizing enzymes.[9] Rapidly regenerating hepatic tissue (such as after partial hepatectomy) is low in glycogen and deficient in ability to metabolize drugs.[10, 11] Newborn animals have low hepatic glycogen levels[12] and have low levels of enzymic drug metabolism.[13] These studies may indicate a possible relationship between hepatic glycogen levels and rates of enzymic drug metabolism: the ability of the liver to lay down glycogen may parallel the ability of the liver to metabolize drugs.

There appears to be a morphological association among the enzymes of hepatic glycogenesis, glycogenolysis, and the microsomal enzymes. Glycogen synthetase (or UDPG transferase) is recovered in hepatic homogenates bound to the particulate glycogen, in the microsomes, and in the soluble fraction.[14, 15] A large portion of the glycogenolytic activity is recovered in the microsomal fraction of liver homogenates.[16]

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SKF 525-A has inhibited certain microsomal enzyme pathways of drug metabolism both *in vivo* and *in vitro*.[17] In this paper we have shown the effect of SKF 525-A on hepatic glycogen levels in relation to inhibition of certain hepatic microsomal enzymes.

METHODS

Male white Holtzman rats, weighing 200 to 250 g, maintained on Wayne lab block, were injected intraperitoneally (i.p.) with SKF 525-A in aqueous solution. For certain experiments adrenal-demedullated Sprague-Dawley rats (obtained from Hormone Assay Imports, Chicago, Ill.) were used. All animals were sacrificed by a blow on the head between 8 and 9 a.m. because the amount of glycogen in the liver changes with time in a 24-hr cycle. A liver slice (0·2 to 0·6 g) was removed as soon as possible after the animal's death and placed in 30% KOH. Hepatic glycogen levels were determined with a phenol–sulfuric acid method.[18] The level of hepatic glycogen was calculated as grams glycogen per gram wet weight liver and then converted to per cent hepatic glycogen. Samples for histological studies were placed in 10% formaldehyde and slides were prepared and examined by the Pathology Department. The remainder of the liver was chilled and used to determine hepatic metabolism of drugs. The conditions of homogenization and incubation as well as concentrations of cofactors and drugs were those used by McLuen and Fouts.[19]

The side chain oxidation of hexobarbital and the *o*-dealkylation of codeine were the metabolic pathways studied. Hexobarbital was determined by the method of Cooper and Brodie.[20] The amount of substrate disappearance was taken as a measure of biotransformation. Morphine was determined by a colorimetric method of Snell and Snell[21] as an estimate of the *o*-dealkylation of codeine.

The rate of enzymic drug metabolism was expressed as micromoles of drug biotransformed per gram wet weight liver per 2-hr incubation. The levels of hepatic glycogen and rates of drug metabolism were expressed as per cent of control values which were given the value of 100% (see Results, Figs. 1, 2, 3). All statistical methods used are described by Snedecor.[22]

Glycogen synthesis. The system for glycogen synthesis contained 1·0 μmole uridine diphosphoglucose, 2·0 μmoles glucose-6-phosphate, 5 μmoles magnesium sulfate, 0·2 μmole ethylenediamine tetraacetic acid (EDTA), 0·4 mg glycogen (Nutritional Biochemicals Corp.), and 0·08 mg glycogen synthetase.[23] The total volume of the incubate was 0·2 ml with isotonic Krebs-Ringer bicarbonate buffer, pH 7·3, as solvent. The glycogen synthetase was prepared from liver homogenized in 0·5 M sucrose–0·005 M EDTA with a Potter glass homogenizer having a plastic pestle. The liver homogenate was spun at 9,000 $\times$ *g* in an angle centrifuge in the cold for 20 min; the supernatant fraction was then spun in a Spinco model L ultracentrifuge at 140,000 $\times$ *g* for 1 hr. The particulate glycogen with bound glycogen synthetase[14] was resuspended in Krebs-Ringer bicarbonate buffer. The glycogen synthesis system was incubated at 37° for 30 min in a Dubnoff metabolic shaker. The reaction was stopped by heating for 1 min in boiling water. The amounts of uridine diphosphate (UDP) formed per gram nitrogen were used as an estimate of glycogen synthesis, according to the method of Cabib and Leloir.[23] The effect of SKF 525-A on the glycogen synthesis system was studied by the addition of the compound to incubation mixtures (see Results, Table 4).

Glycogenolysis. The enzymes of hepatic glycogenolysis are recovered in the supernatant fraction, after centrifugation at 9,000 $\times$ *g*, along with the glycogen.[16]

This supernatant fraction was prepared as previously described under glycogen synthesis. The quantitative assay for hepatic glycogenolytic activity was carried out as follows. The supernatant fractions were incubated at 37° for 30 min in an equal amount of 0·1 M phosphate buffer (pH 7·3). Final volume of all incubation mixtures was 4·0 ml. Glycogen determinations were made on (9000 × *g*) supernatant fractions and incubation mixtures. Glycogenolytic activity was estimated from the difference in glycogen content of aliquots from unincubated *vs.* incubated (9000 × *g*) supernatant fractions. The effect of SKF 525-A on the glycogenolysis system was studied by the addition of the compound to incubation mixtures (see Results, Table 5).

RESULTS

Acute administration of SKF 525-A. A single dose of SKF 525-A (20 mg/kg; Fig. 1; and 40 mg/kg, Fig. 2) was administered prior to sacrifice (animals always killed

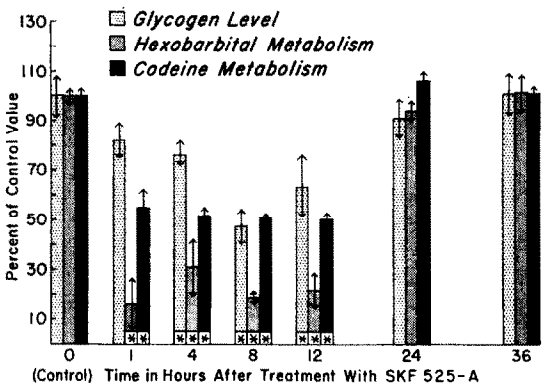

FIG. 1. Effects of SKF 525-A (20 mg/kg i.p.) on hepatic glycogen and drug metabolism. The bars represent mean levels of hepatic glycogen and rates of drug metabolism. The vertical lines (‡) indicate ± standard error ($s\sqrt{n}$, where s = standard deviation and n = total number of animals = 8). The asterisks represent values which are significantly ($P < 0.05$) different from control values according to Student's t distribution.

Control values for this figure were: glycogen, 6·2 ± 1·4% glycogen (wet weight liver); hexobarbital metabolism, 3·50 ± 0·27 μmoles/g wet weight liver/2 hr incubation; codeine metabolism, 1·89 ± 0·16 μmoles/g wet weight liver/2-hr incubation.

between 8 and 9 a.m.). The level of hepatic glycogen was depressed maximally at 8 to 12 hr and returned to control level at 24 to 48 hr after administration of SKF 525-A. The rate of hepatic enzymic metabolism of the two drugs studied was depressed maximally 1 hr after administration of SKF 525-A, but returned to control values between 24 and 48 hr. The hepatic glycogen level and rate of metabolism of the two drugs returned to control values during the same time interval after the administration of a single dose of SKF 525-A.

Chronic administration of SKF 525-A. SKF 525-A (20 mg/kg, Fig. 3) was ad-administered every 12 hr for 5 days prior to determinations of glycogen and rates of drug metabolism. The return of glycogen level and rate of hexobarbital and codeine metabolism to control values occurred at the same time. The effects of SKF 525-A administered chronically were reversible. The rates of recovery of hepatic glycogen and

drug-metabolizing enzyme activity were similar after acute and chronic administration of SKF 525-A.

In our experiments there was no significant stimulation of hexobarbital or codeine metabolism after the administration of single or multiple doses of SKF 525-A. This was in contrast to the reports by Serrone and Fujimoto[24] and may have been caused by differences in experimental design.

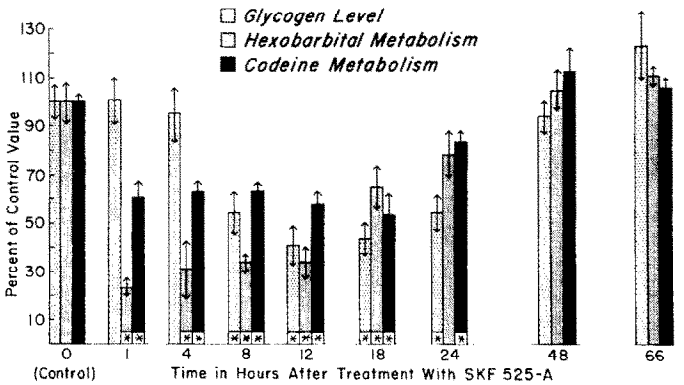

FIG. 2. Effects of SKF 525-A (40 mg/kg i.p.) on hepatic glycogen and drug metabolism (see Fig. 1 for explanation of symbols).

Control values for this figure were: glycogen, 6·6 ± 1·2% glycogen (wet weight liver); hexobarbital metabolism, 3·29 ± 0·82 μmoles/g wet weight liver/2-hr incubation; codeine metabolism, 1·68 ± 0·21 μmoles/g wet weight liver/2-hr incubation.

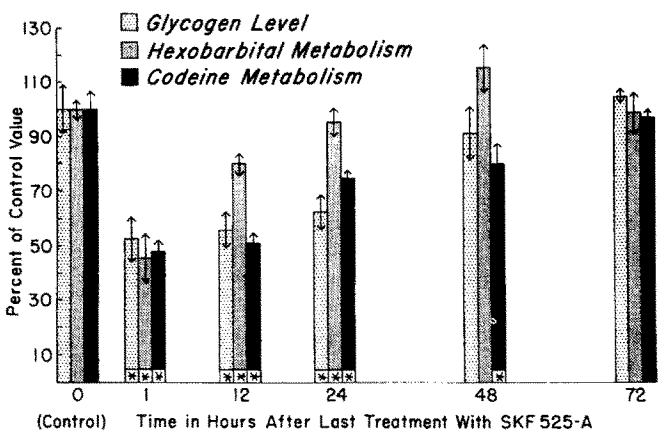

FIG. 3. The effects of chronic SKF 525-A (20 mg/kg i.p. every 12 hr × 10) on hepatic glycogen and drug metabolism (see Fig. 1 for explanation of symbols).

Control values for this figure were: glycogen, 6·7 ± 1·7% glycogen (wet weight liver); hexobarbital metabolism, 3·51 ± 0·29 μmoles/g wet weight liver/2-hr incubation; codeine metabolism, 1·56 ± 0·26 μmoles/g wet weight liver/2-hr incubation.

Effects of SKF 525-A in adrenal-demedullated animals. One mechanism by which SKF 525-A produced these effects on hepatic glycogen might be by the release of epinephrine from the adrenals. We therefore studied the effects of SKF 525-A on glycogen levels and rate of drug metabolism in livers from adrenal-demedullated rats.

The data in Table 1 show that these animals respond to SKF 525-A the same way as do normal rats.

Hepatic glycogen levels in rats given known amount of food. Another mechanism by which SKF 525-A could produce these effects on hepatic glycogen might be by decreasing appetite—*i.e.* inducing starvation. To minimize differences in food consumption between control and treated groups, animals were taken off the regular diet

TABLE 1. ACUTE ADMINISTRATION OF SKF 525-A (20 MG/KG) TO ADRENAL-DEMEDULLATED RATS*

Time after SKF 525-A (i.p.) administration (hr.)	Glycogen level (% wet weight liver)	Hexobarbital metabolism (μmoles/g wet weight liver/2-hr incubation	Codeine metabolism (μmoles/g wet weight liver/2-hr incubation
Control	6·7 ± 1·6	3·50 ± 0·27	1·70 ± 0·36
8	2·3 ± 0·2 $P < 0.001$	0·70 ± 0·20 $P < 0.001$	0·94 ± 0·34 $P < 0.001$
12	0·4 ± 0·2 $P < 0.001$	0·80 ± 0·73 $P < 0.001$	0·63 ± 0·20 $P < 0.001$

* Eight animals were used in each group; Values represent mean ± standard deviation.

TABLE 2. EFFECTS OF SKF 525-A ON HEPATIC GLYCOGEN LEVELS IN RATS GIVEN A KNOWN AMOUNT OF FOOD*

Time after SKF 525-A or water administration, (hr)	Per cent hepatic glycogen per gram (wet weight) liver				
	Control (water injection)	SKF 525-A-treated 40 mg/kg	P	80 mg/kg	P
4	4·8 ± 0·5	4·0 ± 0·6	0·02–0·01	2·3 ± 0·6	<0·001
8	8·3 ± 1·7	4·7 ± 0·5	<0·001	2·9 ± 0·8	<0·001

* Animals were taken off the regular diet and injected i.p. with SKF 525-A (40 or 80 mg/kg) or an equal volume of water; 1 hr later all animals were given 2 ml USP simple syrup (orally). Eight animals were used in each group (mean ± SD).

and treated with SKF525-A, 40 and 80 mg/kg i.p., or an equal volume of water; 1 hr later, all animals were given 2 ml USP simple syrup orally. Table 2 shows that animals given SKF 525-A had significantly less hepatic glycogen than control animals at 4 and 8 hr after drug or water administration.

Hepatic glycogen level in adrenal-demedullated rats given no food after treatment. Table 3 shows other results which indicate that the effects of SKF 525-A on hepatic glycogen are not due just to starvation. For these experiments, control and SKF 525-A-treated rats were taken completely off food. The SKF 525-A-treated animals had significantly lower levels of hepatic glycogen than control animals. Both control and SKF 525-A-treated groups were adrenal-demedullated.

Glycogen synthesis. The effect of SKF 525-A on glycogen synthesis by normal liver fractions is reported in Table 4. Glycogen synthesis was inhibited significantly ($P < 0.05$; Student's *t* test distribution) only by the 10^{-3} M concentration of SKF 525-A.

Glycogen synthetase prepared from animals treated with SKF 525-A did not synthesize glycogen at a slower rate than enzyme from untreated controls (based on micromoles UDP formed in 30 min per gram nitrogen). SKF 525-A had no effect (inhibition or stimulation) on pyruvate kinase or on the chemical assay of UDP.

TABLE 3. HEPATIC GLYCOGEN LEVEL IN ADRENAL-DEMEDULLATED RATS GIVEN NO FOOD AFTER SKF 525-A ADMINISTRATION*

Time after SKF 525-A administration (hr)	Per cent hepatic glycogen per gram (wet weight) liver				
	Control (water injection)	SKF 525-A-treated animals			
		20 mg/kg	P	40 mg/kg	P
4	5·7 ± 2·4	1·6 ± 1·2	<0·001	0·6 ± 0·6	<0·001
8	2·1 ± 0·5	0·3 ± 0·3	<0·001	0·1 ± 0·1	<0·001

* Animals were taken off the regular diet and injected i.p. with SKF 525-A (40 or 80 mg/kg) or an equal volume of water. Eight animals were used in each group (mean ± SD).

TABLE 4. EFFECTS OF SKF 525-A ON SYNTHESIS OF GLYCOGEN BY "GLYCOGEN SYNTHETASE"*

Concentration of SKF 525-A in incubation mixture	μmoles UDP formed per g nitrogen	P
0 (control	65·0 ± 9·2 (8)	
10^{-3} M	45·3 ± 13·1 (8)	<0·001†
10^{-4} M	62·7 ± 11·9 (8)	0·7–0·6
10^{-5} M	69·7 ± 14·6 (8)	0·5–0·4
10^{-6} M	68·0 ± 11·7 (4)	0·7–0·6

* Values represent mean ± SD; numbers of experiments in each group are given in parentheses.
† Value significantly lower than control (P < 0·05).

TABLE 5. EFFECTS OF SKF 525-A ON BREAKDOWN OF GLYCOGEN IN LIVER HOMOGENATES*

Concentration of SKF 525-A in the incubation mixture	Difference in % glycogen after incubation	P
0 (control)	2·5 ± 0·9 (17)	
10^{-3} M	2·8 ± 0·9 (16)	0·4–0·3
10^{-4} M	2·6 ± 1·0 (17)	0·8–0·7
10^{-5} M	2·8 ± 0·9 (17)	0·4–0·3

* Values represent mean ± SD; numbers of experiments in each group are given in parentheses.

Thus SKF 525-A, at any concentration used, did not interfere with the assay of glycogen synthetase activity.

Glycogenolysis. The effect of SKF 525-A on hepatic glycogenolysis in normal liver is shown in Table 5. The addition *in vitro* of SKF 525-A to incubation mixtures did not increase the rate of glycogenolysis significantly ($P < 0·05$).

Homogenates of liver obtained from animals treated with SKF 525-A (40 mg/kg, 3 hr before sacrifice) did not degrade glycogen more rapidly than those from untreated animals.

SKF 525-A at any concentration used (up to 10^{-3} M) did not interfere with the chemical determination of glycogen.

DISCUSSION

This paper investigated the relationship between the amount of hepatic glycogen and the activity of certain microsomal enzymes after administration of SKF 525-A. An abnormally low rate of metabolism of drugs by hepatic microsomes has often been found in liver, which also has very low levels of glycogen.[1] In the newborn animal the ability of the liver to lay down glycogen and metabolize foreign compounds appears at the same time. Both a morphological and physiological relationship between hepatic glycogen and microsomal enzyme activity can be postulated.

Both acute and chronic administration of SKF 525-A lowered hepatic glycogen. After acute administration of SKF 525-A, the activity of the microsomal enzymes metabolizing hexobarbital and codeine decreased more rapidly than did the level of hepatic glycogen. This decrease in the level of hepatic glycogen could indicate that the inhibition of microsomal enzymes alters, or at least occurs, as a result of altering the structure of the SER in such a way as to interfere with the storage of glycogen. The ability of the microsomal enzymes to metabolize hexobarbital and codeine returned at the same time that the hepatic glycogen was replaced.

Possible mechanisms by which SKF 525-A produces its effects on hepatic glycogen include release of epinephrine from the adrenal and effects on the animal which would result in a decreased food intake. The results shown in Tables 1, 2, and 3 seem to indicate that SKF 525-A does not decrease hepatic glycogen by these mechanisms. Thus SKF 525-A still causes marked decreases in glycogen in adrenal-demedullated animals, in animals force fed, and in starved animals.

The glycogen-synthesizing system used in this paper did not demonstrate a significant inhibition of glycogen synthesis by SKF 525-A except at very high concentrations added *in vitro*. The system used to study glycogenolysis did not demonstrate that SKF 525-A added *in vitro* increased the rate of glycogen breakdown. Livers from animals treated with SKF 525-A prior to sacrifice did not have increased rates of glycogen breakdown or decreased rates of glycogen synthesis as compared with the same enzyme systems from control animals. The mechanism responsible for the decrease in levels of hepatic glycogen following administration of SKF 525-A was not established by our experiments.

The effect of SKF 525-A on hepatic glycogen did not appear to be strictly dose related. At 10 mg/kg (i.p.) no significant effect on hepatic glycogen was seen, whereas the effects of 20 mg/kg and 40 mg/kg (i.p.) on glycogen levels were essentially the same. A plateau can be demonstrated in the dose-response curve in the range of 20 to 40 mg/kg (i.p.) of SKF 525-A whether the response is an effect on hepatic glycogen, rate of microsomal drug metabolism, or hexobarbital sleeping times.

Serrone and Fujimoto[24] have reported a biphasic effect of SKF 525-A on hexobarbital sleeping times in mice. SKF 525-A at a dose of 20 mg/kg (i.p.) caused a prolonged hexobarbital sleeping time during the first 24 hr after administration. Between

24 and 48 hr after the administration of SKF 525-A, hexobarbital sleeping times were less than normal.

In our experiments, this delayed stimulation by SKF 525-A of microsomal drug metabolism could not be called statistically significant. A larger number of animals might show that the 10 to 20% increase in rate of microsomal drug metabolism (*in vitro*) that we obtained (Fig. 1, 2) is significant.

Preliminary work in our laboratory indicates that other microsomal enzyme inhibitors such as iproniazid, Lilly 15121 (2,4-dichloro-6-phenylphenoxyethyl dimethylamine), JB516 (β-phenylisopropylhydrazine), and chloramphenicol also decrease levels of hepatic glycogen. Chronic administration of SKF 525-A, which we have shown to result in a decreased hepatic glycogen level, also causes an increase in total hepatic lipid material.[25] We would like to suggest that glycogen depletion and fatty infiltration could be associated with the chronic administration of many compounds which cause inhibition of hepatic microsomal drug-metabolizing enzymes.

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