Hepatic Glycogen Concentration and Hexobarbital-Sleeping Time In Mice. (30789)

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Various factors, such as starvation(1), alloxan diabetes(2), and epinephrine administration(3), are known to reduce the concentration of liver glycogen and at the same time result in inhibition of certain drug-metabolizing enzymes of liver microsomes. In addition, the liver microsomal enzyme inhibitor SKF-525A (β diethylaminoethyl diphenylpropyl acetate HCL), when administered to rats, produces a reduction in liver glycogen concentration and a decreased ability to metabolize hexobarbital(4). It has been postulated, therefore, that the activity of liver microsomal enzymes is related to the concentration of liver glycogen(4). It is possible that the effect of microsomal enzyme inhibitors is mediated through a reduction in the concentration of liver glycogen.

The present study investigated the relationship between the concentration of liver glycogen and hexobarbital-sleeping time in mice as influenced by the microsomal enzyme inhibitors, chlorcyclizine and SKF-525A.

Methods. Swiss Webster male albino mice (24-28 g) were used throughout and received 25 mg/kg of chlorcyclizine or SKF-525A either as a single dose or twice a day for 3 days by intraperitoneal injection. Control animals received an equivalent volume of saline equal to 0.1% of the body weight.

Hepatic glycogen concentration and hexobarbital-sleeping time were determined one hour after a single injection of SKF-525A or chlorcyclizine, or 18 hours after the last injection when treated twice a day for 3 days. Glycogen concentration was determined by the method of Montgomery(5), and all analyses were performed in triplicate. Animals were sacrificed for glycogen assay between 8:30 and 9:30 a.m. to minimize variations in glycogen content.

Sleeping time was evaluated after intravenous injection of 80 mg/kg of hexobarbital. Sleeping time is defined as the interval between time of injection and return of the righting reflex. The righting reflex was considered to have returned when the animal could right itself 3 times within 30 seconds.

All data were analyzed by means of the analysis of variance or the "t" test for the difference between means with confidence limits set at 95%.

Results. Effect of a single injection of chlorcyclizine or SKF-525A on liver glycogen and hexobarbital-sleeping time. A single injection of chlorcyclizine or SKF-525A to fed mice one hour prior to sacrifice did not alter the concentration of hepatic glycogen (Table I). However, inhibition of hexobarbital metabolism produced by chlorcyclizine and SKF-525A is indicated by a marked prolongation of sleeping time. The sleeping time of the chlorcyclizine-treated mice was increased 90% above that of the control group and that of the SKF-525A-treated group, 210% above the control group. The results suggest that these agents produce an acute inhibition of the metabolism of hexobarbital without a concomitant effect on liver glycogen.

Effect of repeated injections of chlorcyclizine or SKF-525A on liver glycogen and hexobarbital-sleeping time. The repeated administration of many compounds, including chlorcyclizine and SKF-525A is known to enhance the rate of metabolism of many drugs, including hexobarbital, presumably by increasing the activity of liver microsomal enzymes(6). If the activity of liver microsomal enzymes is related to the concentration of liver glycogen as previously suggested(4), then it was possible that a decreased hexobarbital-sleeping time, produced by repeated injections of chlorcyclizine or SKF-525A, might be associated with an elevated liver glycogen concentration. No such correlation was found. Although hexobarbital-sleeping time was reduced 65% and 76% by chlorcyclizine and SKF-525A respectively, liver glycogen concentration was not significantly altered by either drug (Table II). The only other effect noted was

TABLE I. Effect of a Single Dose of SKF-525A and Chlorcyclizine on Hexobarbital-Sleeping Time and Liver Glycogen Concentration.

Treatment	Liver, % body wt ± S.E.M.	Liver glycogen, mg/g ± S.E.M.	Sleeping time, min ± S.E.M.
Saline	6.42 ± .24	46.14 ± 6.44 (5)	31.9 ± 1.6 (11)
Chlorcyclizine	5.75 ± .93	41.94 ± 6.00 (5)	59.9 ± 4.1 (10)
SKF-525A	5.41 ± .17	38.03 ± 4.19 (5)	99.0 ± 6.3 (8)

Numbers in parentheses indicate No. of animals in each group.

TABLE II. Effect of Repeated Injections of SKF-525A and Chlorcyclizine on Hexobarbital-Sleeping Time and Liver Glycogen Concentration.

Treatment	Liver, % body wt ± S.E.M.	Liver glycogen, mg/g ± S.E.M.	Sleeping time, min ± S.E.M.
Saline	6.60 ± .10	31.34 ± 2.74 (11)	34.6 ± 2.7 (22)
Chlorcyclizine	9.01 ± .27	22.26 ± 3.47 (12)	12.4 ± 1.0 (15)
SKF-525A	6.85 ± .14	24.60 ± 3.07 (10)	8.3 ± .4 (14)

Numbers in parentheses indicate No. of animals in each group.

TABLE III. Effect of a Single Dose of SKF-525A and Chlorcyclizine on Hexobarbital-Sleeping Time and Liver Glycogen Concentration Following 48-Hour Starvation.

Type	Treatment	Liver, % body wt ± S.E.M.	Liver glycogen, mg/g ± S.E.M.	Sleeping time, min ± S.E.M.
Fed	Saline	6.10 ± .12	28.35 ± 3.35 (8)	36.1 ± 3.5 (11)
Starved	Saline	5.21 ± .26	7.34 ± .85 (10)	69.5 ± 10.7 (16)
"	Chlorcyclizine	5.33 ± .21	8.96 ± 1.57 (9)	152.1 ± 13.6 (13)
"	Saline	5.31 ± .33	6.74 ± 1.41 (10)	76.7 ± 10.2 (15)
"	SKF-525A	5.16 ± .23	7.92 ± 2.75 (8)	251.4 ± 9.2 (11)

Numbers in parentheses indicate No. of animals in each group.

a 36% increase in liver weight in the chlorcyclizine-treated group. A similar effect with chlorcyclizine has previously been reported by Welch and Coon(7).

Effect of chlorcyclizine or SKF-525A on liver glycogen and hexobarbital-sleeping time of starved mice. It is known that starvation is accompanied by a reduction in liver glycogen concentration and a reduction in the activity of certain drug-metabolizing enzymes of liver microsomes(1). If agents which inhibit the activity of liver microsomal enzymes do so by effecting a reduction in glycogen concentration, then an abnormally low glycogen level, produced by starvation, might be expected to abolish or reduce the inhibitory effect of chlorcyclizine or SKF-525A on hexobarbital metabolism.

Starvation alone produced an approximate 75% reduction in liver glycogen concentration and a 2-fold increase in sleeping time (Table III). Chlorcyclizine and SKF-525A, when administered to starved mice, produced no further change in glycogen concentration. However, the effects on sleeping time produced by both drugs were marked. When compared to the saline-treated starved control group, the sleeping time of the starved chlorcyclizine- and SKF-525A-treated groups was increased 120% and 230%, respectively.

Discussion. In studies in which liver glycogen has been reduced by starvation(1), alloxan diabetes(2), epinephrine(3), and SKF-525A(4), the activity of certain drug-metabolizing enzymes of liver microsomes is also reduced. Other studies have demonstrated that decreased microsomal enzyme activity is not always correlated with reduced liver glycogen concentration(8). Nevertheless, it has been suggested that the activity of certain drug-metabolizing enzymes of liver microsomes is in some way related to the concentration of liver glycogen since rats treated with SKF-525A manifested a reduction in liver glycogen concentration which was associated with an inhibition in the metabolism of codeine

and hexobarbital(4). Conceivably, agents which inhibit the activity of liver microsomal enzymes do so by reducing the concentration of liver glycogen.

In the present study, using hexobarbital-sleeping time as an indirect measure of hexobarbital metabolism, the effects of chlorcyclizine and SKF-525A on hexobarbital metabolism could not be attributed to reduced liver glycogen concentration. Neither chlorcyclizine, which has previously been demonstrated to prolong hexobarbital-sleeping time and to inhibit the metabolism of certain drugs (9), nor SKF-525A had any effect on liver glycogen concentration one hour after injection although both agents markedly prolonged sleeping time.

In contrast to the potentiation of hexobarbital action produced by a single injection of SKF-525A or chlorcyclizine, administration of either agent twice a day for 3 days resulted in an increase in the rate of hexobarbital metabolism as indicated by a decrease in sleeping time. However, there was no associated change in liver glycogen concentration. Therefore, both chlorcyclizine and SKF-525A increased the rate of hexobarbital metabolism in fed mice without affecting liver glycogen.

The experiments performed with starved mice demonstrated that starvation alone prolongs sleeping time and reduces liver glycogen concentration. In our experiments sleeping time was doubled as a result of starvation. These results are in agreement with those reported by Dixon *et al*(1). However, when chlorcyclizine or SKF-525A was administered to starved mice, in which glycogen concentration was depressed to approximately 30% of control levels, sleeping time was further prolonged without a further reduction of liver glycogen concentration. In addition, it was noted that in starved as well as fed mice, those treated with a single dose of SKF-525A slept 1.6 times as long as those treated with chlorcyclizine.

Summary. The results of the present study demonstrated that the alterations of hexobarbital metabolism produced by chlorcyclizine and SKF-525A are independent of the effects of these agents on liver glycogen and do not support the concept of a relationship between liver glycogen levels and the activity of drug-metabolizing enzymes of liver microsomes.

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