INDUCTION OF HEPATIC CARISOPRODOL-METABOLIZING ENZYME BY PRETREATMENT WITH SOME NEUROPSYCHOTROPIC DRUGS IN RATS

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In previous works, an increased metabolism of meprobamate in rats pretreated with some drugs were reported.

The increased in vivo metabolism of meprobamate might be due to increased breakdown of the drug by the hepatic microsomal enzyme (1, 2). In the work reported here the induction of hepatic carisoprodol metabolizing enzyme by the pretreatment with phenobarbital, phenaglycodol and Doriden was studied by determining the enzyme activity using liver slice preparation, and the possible difference in sensitivity of the induction of the enzyme to the inducers among rats of different age was also studied.

MATERIALS AND METHODS

Throughout this work, rats of Sprague-Dawley strain, breded in our Institute were used. Experiments in vitro were carried out by using female rats, weighing about 60 g, and the determination of the duration of paralysis were also carried out by using young female rats, weighing about 160 g. In some experiments adult female rats, weighing about 280 g and young female rats, weighing about 160 g and also immature female rats, weighing about 80 g, were used.

The determination of carisoprodol concentrations in vitro and in vivo were carried out according to the method described in the previous report (3). The enzyme activity was determined by measuring the metabolized carisoprodol during two hours of incubation with 500 mg liver slices which were suspended in 6.0 c.c. of the Krebs Phosphate Ringer (pH 7.4) with addition of 0.2 c.c. of substrate (400 µg) at 37°C in air-atomosphere and after finishing the incubation the reaction mixture was homogenized and 2 c.c. of the homogenate were used for the determination. In vivo experiments carisoprodol was suspended in 1% solution of methylcarboxycellulose and injected intraperitoneally in doses of 150 mg/kg.

Duration of paralysis produced by carisoprodol was determined by the duration of loss of the righting reflex (room temperature 20-22°C). Phenobarbital (70-90 mg/kg) was dissolved in distilled water and phenaglycodol (100-130 mg/kg) and Doriden (70-80 mg/kg) were suspended in 1% solution of carboxymethylcellulose and all drugs were injected intraperitoneally.

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RESULTS

Fig. 1 shows that the degradation of carisoprodol by liver slices was about 221 µg/g/2hrs but the enzyme activity begins to increase 12 hours after the administration of the inducing drugs, and the maximum degradation rate was found to be at 36-48 hours after the injection of the drugs. The maximum enzyme activities in the pretreated rats were following: the phenobarbital pretreated rats was 568 µg/g/2hrs, the phenaglycodol pretreated rats was 446 µg/g/2hrs, the Doriden pretreated rats was 418 µg/g/2hrs.

The increased enzyme activity in the pretreated animals was disappeared 96-120 hrs after the pretreatments.

Fig. 2 also demonstrated that the duration of carisoprodol paralysis in normal rats was 128 min. On the other hand, the durations of the paralysis 48 hours after the pretreatments with the drugs were following: 18 min in the phenobarbital pretreated rats, 38 min in the phenaglycodol pretreated rats and 49 min in the Doriden pretreated rats.

From Fig. 1 and Fig 2 it was disclosed that the variations of duration of the paralysis are negative correlation to that of the hepatic enzyme activities in the pretreated rats. The increase in the degradation of carisoprodol and decrease in the duration of the paralysis by the pretreatment with phenobarbital, phenaglycodol and Doriden were antagonized by combined injection of 200 mg/kg ethionine which itself does not modify the enzyme activity (Table 1).
During the experiments a remarkable difference was observed in the degradation of carisoprodol between different aged rats and also in sensitivity of the induction of the hepatic enzyme activity to the inducers among rats of different age.

Table 2 shows a difference in the metabolism in vitro of carisoprodol and also in the sensitivity to the inducer among rats of different age.

**Table 2.** Difference in metabolism of carisoprodol and in sensitivity to the inducers between immature rats and adult rats.

<table>
<thead>
<tr>
<th>Pretreatment</th>
<th>No. of animals</th>
<th>Enzyme activity (µg/g/2hrs)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Adult controls</td>
<td>8</td>
<td>102 ± 5.8</td>
<td></td>
</tr>
<tr>
<td>2) Phenobarbital pretreated</td>
<td>6</td>
<td>182 ± 7.3</td>
<td>1) - 2) + 78</td>
</tr>
<tr>
<td>3) Phenaglycodol pretreated</td>
<td>4</td>
<td>146 ± 6.5</td>
<td>1) - 3) + 41</td>
</tr>
<tr>
<td>4) Immature controls</td>
<td>10</td>
<td>223 ± 8.0</td>
<td>1) - 4) + 113</td>
</tr>
<tr>
<td>5) Phenobarbital pretreated</td>
<td>8</td>
<td>581 ± 12.3</td>
<td>4) - 5) + 156</td>
</tr>
<tr>
<td>6) Phenaglycodol pretreated</td>
<td>6</td>
<td>438 ± 11.5</td>
<td>4) - 6) + 96</td>
</tr>
</tbody>
</table>

Phenobarbital and phenaglycodol were injected 48 hours before the sacrifice.

The carisoprodol metabolism by liver slices of normal adult rats was 102 µg/g/2hrs, and that of normal immature rats was 223 µg/g/2hrs. The degradation of carisoprodol was increased 78% in adult rats and 156% in immature rats by the pretreatment with phenobarbital and also 41% in adult rats and 96% in immature rats by phenaglycodol.

It is a very interesting problem that whether or not the observed remarkable difference in the metabolism of carisoprodol and in the sensitivity to the inducers among rats of different age can be observed only in the slice preparation and whether it can be observed in vivo metabolism of carisoprodol or not.

Fig. 3 shows that a faster metabolism of carisoprodol and a higher sensitivity to the inducers were observed in immature rats than in young and adult rat as those observed by using the slice preparation. For example, “metabolic half life” of carisoprodol in di-
Different aged normal rats and phenobarbital pre-
treated rats were following:

- Normal immature rats: 138 min
- Normal young rats: 205 min
- Normal adult rats: 354 min
- Pretreated immature rats: 33 min
- Pretreated young rats: 52 min
- Pretreated adults rats: 164 min

**DISCUSSION**

Recently the induction of hepatic drug-
metabolizing enzymes by the pretreatment with
some drugs was reported by some authors (1-10).

The inducible drug-metabolizing enzymes exist
in microsome of liver and require TPNH and
oxygen for their activity and they are inhibited
by SKF 525 A (2, 3, 5, 8, 9, 11-13).

The recent work demonstrates that enzyme
responsible for metabolism of carisoprodol exists
also in microsome of liver and requires TPNH
and oxygen for its activity and the activity is in-
hibited by SKF 525 A (3, 14).

Carisoprodol is a derivative of meprobamate
and the enzyme responsible for the metabolism of the two drugs are inducible hepatic
microsomal enzymes, but the enzyme responsible for the metabolism of carisoprodol may
be different from the enzyme responsible for the metabolism of meprobamate, because
the former shows a remarkably different activity between male and female rats, but the
latter does not (15, 16).

But the results obtained in this study show the enzyme responsible for metabolism
of carisoprodol is an inducible enzyme and the induction of the enzyme is inhibited by
the combined injection of ethionine and an ineffectiveness of direct addition of the indu-
cers on the enzyme activity suggest that the nature of the enzyme action may be adap-
tive as same as that of meprobamate.

The time required for an increase of enzyme activity after the injection of the in-
ducer may represent a time for adaptation.

The variation of the activity of hepatic drug-metabolizing enzymes in relation to age
have been only reported by Brodie et al. on the newborn animals (17). According to the
results of Brodie et al. the newborn rat does not show enzyme activity and increase of
the enzyme activities appears 2-3 days after, and the rats of 21 days old showed the same
enzyme activity as that of adult rats.

Our results suggest that rats of 60-80 g body weight (30-40 days old) have the highest
ability in the degradation of carisoprodol and the younger rats have a higher sensitivity to the inducers than older ones.

The possibility whether the different ability of degradation of the drug and the different sensitivity to the inducers are limited only to metabolism of carisoprodol or not, is now being studied in this laboratory.

The mechanism by which the higher sensitivity to the inducers of the liver of younger rats is not yet clear. Regenerating liver does not show a higher sensitivity to the inducers in any stage of regeneration, therefore a higher sensitivity to the inducers may be under a control of some humoral factors of animals. It is very interesting to note that the activity of hepatic tryptophane peroxidase is increased by the administration of tryptophane and returns to normal level within 24 hours (18), whereas, the variation of the enzyme responsible for metabolism of carisoprodol begins slowly and returns to normal level 96-120 hours after the pretreatment.

Those results suggest that the turnover rates of the hepatic drug-metabolizing enzyme is slow and that of hepatic tryptophane peroxidase is quick.

Also the activity of the hepatic carisoprodol-metabolizing enzyme was increased not only by pretreatments with phenobarbital, phenaglycodol and Doriden, but also by pretreatments with other inducers; such as meprobamate, carisoprodol itself, chlorpromazine, thiopental, pentobarbital, mysoline, diphenyl-hydantoin, and coramin, etc.

**SUMMARY**

The degradation of carisoprodol by liver slices was increased by the pretreatments with phenobarbital, phenaglycodol and Doriden. The increase of the enzyme activity began 12 hours after the injection of the inducers and the maximum increase of the enzyme activity (about 2.5-fold) was observed 48 hours after and 96 hours after a little increased activity observed.

In contrast to the increase of enzyme activity the shortened duration of paralysis produced by carisoprodol was observed after the pretreatment with the inducers and also the maximum diminution (about 85%) was found 48 hours after the pretreatments. The induction of the enzyme responsible for metabolism of carisoprodol by the inducers was inhibited by combined injection of ethionine which itself does not affect on the enzyme activity of normal rats.

The higher activity of the hepatic enzyme responsible for carisoprodol metabolism and the higher sensitivity in the induction of the hepatic enzyme to the inducers in immature rats than in young and adult rats were also reported.

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