METABOLIC DIFFERENCES OF STRYCHNINE IN THE RAT IN RELATION TO SEX

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In the previous paper, the remarkable difference in strychnine toxicity between adult female and adult male rats was reported (1).

This sex difference is observed in cases of intraperitoneal, subcutaneous or oral administration of the drug but not by intravenous injection. After pretreatment with SKF 525 A such sex difference also disappeared.

The different capacities of breakdown of strychnine by liver of female and male rats were supposed to account for such sex difference. It is worth while for noticing that such remarkable sex difference in strychnine toxicity after intraperitoneal injection of the drugs takes place within 10 minutes.

It has been generally considered that such short time is not enough to produce marked difference through metabolic processes, and a different sensitivity of central nervous system in both sexes was considered to be a responsible factor (2, 3).

In recent time, the authors have demonstrated that there is a marked decrease of strychnine toxicity through an increase of strychnine metabolism by pretreatment (48 hours before) with phenobarbital, phenaglycodol, thiopental and glutethimide (4-6).

These results suggested a rapid “in vivo” metabolism of strychnine which could produce a marked difference in the toxicity by a modification of metabolic rate.

In this paper, the possibility of the presence of such sex difference in metabolism of strychnine between both sexes of rats was studied.

MATERIALS AND METHODS

Both sexes of Sprague-Dawley strain rats were used. The toxicity of strychnine was evaluated by determination of 50% convulsion doses and 50% lethal doses, calculated according to the methods of Litchfield and Wilcoxon (7).

Strychnine metabolism was determined by measuring the metabolized strychnine during an incubation of 2 hr.

The rats were killed by decapitation and the liver immediately removed and sliced with a microtome or homogenized with a Potter-Elvehjem type homogenizer adding 2 volumes of KCl 1.15%. Sliced livers (500 mg) were suspended in a Warburg flask which
SEX DIFFERENCE ON STRYCHNINE

contained 6 ml of Krebs phosphate buffered Ringer (pH 8.2) and 0.2 ml of 532 μg of strychnine sulphate (final concentration 2×10⁻⁴ M) and incubated in an atmosphere of oxygen at 37°C for 2 hr with shaking. At the end of the incubation period the reaction mixture was homogenized and 2 ml of the homogenate were used for the determination of strychnine concentration.

In the experiments with the microsomal preparation, the nuclei and mitochondria were sedimented by centrifugation of the homogenate at 8,500 g for 15 min, and the microsomal preparation (5.0 ml) was made up adding the followings to 2 ml of the supernatant: 0.1 ml of 20 M glucose-6-phosphate, 0.4 μM TPN, 50 μM nicotinamide, 75 μM MgCl₂ and 1 M KCl, and more 2.3 ml of 0.1 M phosphate buffer and 0.2 ml of the substrate.

In some experiments the microsomes were separated by centrifugation at 10,500 g for 60 min from the microsome-containing supernatant, which was obtained after the centrifugation at 8,500 g for 15 min.

The microsomal preparations were incubated in 25 ml Erlenmeyer flasks which were shaken in air at 37°C and 2 ml of the reaction mixture were used for the determination.

The remained strychnine concentration was determined by reading the ultraviolet absorption with a spectrophotometer.

Add 25 ml of heptane containing 1.5 % isoamylalcohol to a glass-bottle of 60 ml volume containing 2 ml of the homogenate or of the reaction mixture and 2 g of NaCl and 0.5 ml of 1 N-NaOH. Shake for 45 min and centrifuge for 5 min. Transfer 20 ml of the heptan phase to another glass-bottle containing 4 ml of 0.1 N-HCl. Shake for 3 min and then centrifuge for 5 min. Transfer about 3 ml of the aqueous phase to a quartz cuvet and determine the optical density at 255 mμ and 280 mμ in Beckman spectrophotometer.

RESULTS

1) Strychnine toxicity in rats of both sexes after administration through different routes

Strychnine toxicity in both sexes of mature rats after administration through different routes was summarized in Table 1.

The convulsions by strychnine administration through various routes appear about 9 min, 25 min, and 6 sec, respectively after the intraperitoneal, subcutaneous and intravenous injections.

The sex difference in strychnine toxicity was marked according to delayed onset of the convolution. Sex difference in LD₅₀ of strychnine by intraperitoneal injection was 1.74 times while 2.22 times by subcutaneous injection and, when strychnine was injected subcutaneously 0.01% adrenaline solution, the onset of strychnine convulsions was about 4 hr later and the sex difference was 2.93 times. On the contrary, there was no sex difference after the intravenous injection.

The latent times of strychnine toxicity may represent the times in which the sex difference is produced through a different rate of breakdown of strychnine by the liver.
enzymes. The sex difference was only observed in adult rats and it was not observed in immature rats nor in adult guinea pigs and mice.

2) "In vitro" metabolism of strychnine by the livers of rats of both sexes

"In vitro" metabolism of strychnine by livers of both sexes of different aged rats were shown in Fig. 1.

Strychnine metabolisms in the hepatic microsomal and slice preparations of newborn rats were very little and the metabolism increases according to aging up to 30 days, but the sex difference was not observed before 40 days.

The ages of maximal metabolic capacity of strychnine of female and male rats were 30 days old and 50 days old respectively.

It is of interest that the younger rats have higher enzyme activity than adult rats. The sex difference becomes apparent in 50 days old rats and difference in enzyme activity was about 2.2–2.5 times and this proportion remained in aging until 250 days old.

Fig. 2 demonstrated the remarkable me-

### TABLE 1. Strychnine toxicity in rats through different routes of administration.

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Sex</th>
<th>No. of animals</th>
<th>CD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Difference</th>
<th>LD&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) i.p.</td>
<td>f</td>
<td>143</td>
<td>1.50 (1.36–1.65)</td>
<td>1.62 (1.48–1.77)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>134</td>
<td>2.61 (2.46–2.89)</td>
<td>2.82 (2.59–3.09)</td>
<td>1.74</td>
<td></td>
</tr>
<tr>
<td>3) s.c.</td>
<td>f</td>
<td>36</td>
<td>1.67 (1.54–1.72)</td>
<td>1.81 (1.65–1.99)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>40</td>
<td>3.72 (3.33–4.17)</td>
<td>4.01 (3.55–4.56)</td>
<td>2.22</td>
<td></td>
</tr>
<tr>
<td>5) s.c.</td>
<td>f</td>
<td>38</td>
<td>2.72 (2.52–2.94)</td>
<td>2.80 (2.61–3.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>40</td>
<td>7.8 (6.4–9.4)</td>
<td>8.2 (6.8–9.9)</td>
<td>2.93</td>
<td></td>
</tr>
<tr>
<td>7) i.v.</td>
<td>f</td>
<td>58</td>
<td>0.50 (0.45–0.56)</td>
<td>0.57 (0.51–0.62)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>m</td>
<td>44</td>
<td>0.51 (0.46–0.57)</td>
<td>0.57 (0.51–0.63)</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

Female rats of 220 g and male rats of 280 g were used.

The subcutaneous injections were made in abdominal regions.

The intravenous injection were made in left femoral vein in volume of 1 ml/kg.

The strychnine solution was made up with 0.1% adrenaline solution.

The numerals in the brackets after CD<sub>50</sub> and LD<sub>50</sub> indicate upper and lower limit with 95% probability.

![Fig. 1](image-url)
Sex difference also in an early stage of the incubation. It was also demonstrated that adult guinea pigs and mice like immature rats, had no sex difference in the strychnine metabolism. These results are in accord with those of the pharmacological experiments.

3) Effect of SKF 525 A on toxicity and metabolism of strychnine

SKF 525 A* was a potent inhibitor of metabolism of some drugs (8–10).

In this experiment, possible disappearance of sex difference in toxicity and metabolism of strychnine was examined.

Table 2 shows that SKF 525 A increases strychnine toxicity and inhibits its metabolisms, and after treatment with SKF 525 A the toxicity and metabolism become similar in both sexes.

These results indicate that there are no sex differences concerning sensitivity to strychnine nor passage of the drug in central nervous system.

![Fig. 2. Metabolisms of strychnine by incubation of different periods.](image)

**Fig. 2.** Metabolisms of strychnine by incubation of different periods.

- a) Strychnine metabolism in the liver microsomal preparation.
- b) Strychnine metabolism in the liver slice.

Experiments were on female and male rats, weighing 200 g and 270 g respectively.

<p>| Table 2. Effect of SKF 525 A on toxicity and metabolism of strychnine on female and male rats. |
| --- | --- | --- | --- | --- |</p>
<table>
<thead>
<tr>
<th>Sex</th>
<th>SKF 525 A treatment</th>
<th>No. of animals</th>
<th>L.D_{50}</th>
<th>Strychnine metabolism in slices (µg/g liver/2h.) in microsome</th>
</tr>
</thead>
<tbody>
<tr>
<td>f</td>
<td>-</td>
<td>62</td>
<td>1.61 (1.48–1.76)</td>
<td>208±7.8 (8)</td>
</tr>
<tr>
<td>f</td>
<td>+</td>
<td>48</td>
<td>1.33 (1.25–1.42)</td>
<td>32±4.9 (6)</td>
</tr>
<tr>
<td>m</td>
<td>-</td>
<td>54</td>
<td>2.82 (2.54–3.12)</td>
<td>421±11 (8)</td>
</tr>
<tr>
<td>m</td>
<td>+</td>
<td>48</td>
<td>1.38 (1.31–1.46)</td>
<td>47±7.1 (6)</td>
</tr>
</tbody>
</table>

Experiments were on female and male rats, weighing about 200 g and 250 g respectively.

50 mg/kg of SKF 525 A were injected intraperitoneally 50 min before injection of strychnine.

In the “in vitro” metabolism of strychnine, SKF 525 A was added at the concentration of 1×10^{-4}M.

The numerals in the bracket after standard errors represent number of the determination.

4) Influence of sex hormones on metabolism of strychnine

Some works showed that metabolic difference of the barbiturates in rats of both

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* SKF 525 A was kindly supplied by Dr. H.E. Duell (Smith Klein & French Laboratories, Philadelphia).
sexes were modified by pretreatment with sex hormones and our recent work also demonstrated a modification of metabolism of carisoprodol in both sexes (11-16).

The rats were castrated 25 days before and treated with female and male hormones for 10 days before sacrifice.

The castration caused a decrease of the metabolic activity and an increase of the toxicity of male rats, but it did not cause any modifications in female rats.

The treatment with male hormone increased the enzyme activity and decreased the toxicity in both sexes of the castrated rats; on the other hand, the treatment with female hormone did not modify the enzyme activity (Fig. 3).

Treatment with 4-chlorotestosterone, which has a potent anabolic action with a very weak androgenic action, also increased the liver enzyme activity and decreased the toxicity (17).

5) Studies on mechanism of the sex difference in strychnine metabolism

The experiments were carried out for detecting a localization of factors which determine the sex difference in strychnine metabolism. The supernatant obtained

| Table 3. Effect of exchange between microsomes and supernatant obtained from liver of female and male rats on strychnine metabolism. |
|---|---|---|---|
| Micr. | Supr. | No. | Strychnine metabolism (µg/g/2hr) |
| female | female | 4 | 102 ± 5.8 |
| female | male | 4 | 96 ± 4.9 |
| male | female | 4 | 223 ± 8.2 |
| male | male | 4 | 258 ± 9.1 |
| male + female | male - female | 4 | 181 ± 6.1 |

Experiments were on female and male rats, weighing 180 g and 250 g respectively. The microsomes obtained by ultracentrifugation of the supernatant, obtained by centrifugation at 8,500 g for 15 min, at 10,500 g from 60 min and supernatant was separated by a decantation.

In the experiment 5) the same volume of microsomes and supernatant of both sexes were mixed.
from male rats was added to the microsomes obtained from female rats or vice versa, and the microsomes of female rats and of male rats were mixed.

The results are shown in Table 3. The results suggested that the factor which might have accounted for the sex difference is only localized in the microsomes and the sex difference is not due to the presence of an activator nor of an inhibitor in the supernatant fraction.

In the experiment 5 the incubation mixture, which consisted from the same volume of microsomes and supernatant of both sexes, has about average value of experiment 1 and 4.

Though TPNH, instead of the supernatant, was added to the microsomes obtained from female or male rats, the similar results were obtained.

DISCUSSION

The results of the present study show that the sex difference in the toxicity of strychnine depends upon a high metabolic activity of the liver microsomes of adults male rats. After the pretreatment with SKF 525 A or the intravenous injection the sex difference was not observed. These results indicate that there is no difference in sensitivity of central nervous system to strychnine and no difference in penetration of the drug into central nervous system of female and male rats.

The sex differences in the pharmacological effects of the barbiturates and their metabolisms in rats are well known (11-14). Recently authors also observed a remarkable sex difference in the paralytic effect of carisoprodol and its metabolism (15). It is very interesting to note that there are following analogies among the metabolism of pentobarbital, hexobarbital, carisoprodol and strychnine (4-6, 10, 18-30):

1) The enzymes responsible for their metabolism were found only in the microsomes of liver.
2) The enzymes require TPNH and O2.
3) The presence of a sex difference was found only in adult rats.
4) The enzyme activities are increased by pretreatment with phenobarbital, phenacylglycol, or glutethimide.
5) The enzyme activities are inhibited by SKF 525 A, Lilly 32391, MG 3062, marsilid and isoniazid.

A question arisen from the above-mentioned similarities is whether the enzymes which metabolized hexobarbital, strychnine and carisoprodol are the same or not.

Our unpublished results indicate that they might be different because they showed different sensitivity after hepatectomy or ligation of common bile duct or in inhibition by SKF 525 A, Lilly 32391, MG 3062, marsilid and isoniazid.

Anabolic action of the sex hormone was considered a factor which produced the sex difference. The mechanism of the anabolic hormones is not yet clear. But an increase of the enzyme protein was supposed.

But the anabolic hormones can not produce an increase of the enzyme activity of
all microsomal drug-metabolizing enzymes, they produce the effects only on some drug-metabolism.

It is also worth while to note that the anabolic hormones can increase the enzyme activities only in rats, but the inducing drugs are active not only in rats, but also in mice and in guinea pigs (31).

**SUMMARY**

A marked sex difference in the toxicity of strychnine was demonstrated, and the greater metabolic capacity of liver microsomes of male rats was ascribed to be a responsible factor. The castration and the treatment with anabolic hormones caused a modification of the strychnine toxicity and metabolism.

The greater metabolic activity of male liver may be due to an anabolic effect of male hormone on liver microsomes.

The similarities between hexobarbital and strychnine metabolism were discussed.

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**REFERENCES**

4) KATÔ, R.: *Arzneimittel Forsch.* 11, 797 (1961)
14) EDGERN, R. A.: *Experientia* 13, 86 (1957)